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EFFECTS OF SOME INGESTED INSECTICIDES ON THE MIDGUT WALL OF THE SOUTHERN ARMYWORM LARVA[1]

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INTRODUCTION

Although the specific action of insecticides on insects has received little attention in the past, the idea is gaining favor that in this field there are many problems that warrant special consideration. Further knowledge might prove useful in the selection, improvement, and application of methods for insect control. A review of the physical and chemical effects of poisons on insect tissues, cells, and secretions is given by Trappmann (*13*).[2] The histopathological effects on the midgut wall of the southern armyworm larva (*Prodenia eridania* (Cram.)) of certain poisons commonly used as insecticides have been studied by the writer in connection with an investigation of the normal anatomy of the alimentary canal, and are described in this paper.

MATERIALS AND METHODS

The poisons used were a high-grade acid lead arsenate, a fine fraction of paris green separated from a commercial lot, an average commercial grade of calcium arsenate, calcium arsenite,[3] arsenic trioxide, a finely divided commercial barium fluosilicate (20 percent silica), reagent-quality sodium fluoride, synthetic sodium fluoaluminate containing some uncombined aluminum oxide, chemically pure rotenone, and a high-grade commercial phenothiazine.

The larvae were reared on living turnip plants in a greenhouse insectary or on fresh turnip foliage in an incubator at 27° C. Actively feeding early sixth instars were removed from the plants and conserved for several hours on fresh, clean leaves in a clean glass cage, in order that any hard objects that might be present in the lumen of the gut would be eliminated. They were then held without food for several hours to induce hunger.

Lethal or unusually large doses of the poisons were fed to the larvae in turnip-leaf or sweetpotato-leaf sandwiches. The larvae were then held at 22°–27° C., and after different intervals were killed by immersion in hot water (60°–70°). The gut-wall tissue was prepared by the paraffin method for a microscopic study of the tissues and cells. Fixation was with Bouin's picro-formol-acetic. Sections 5 to 10 microns thick were cut in both the transverse and the longitudinal planes, stained with Ehrlich's haematoxylin, and counterstained with eosin or erythrosin. Similar preparations of larvae that had not ingested poisons were used for comparison.

[1] Received for publication April 13, 1940.

[2] Italic numbers in parentheses refer to Literature Cited, p. 328.

[3] This product contained 45.7 percent As_2O_3, equivalent to 85 percent $Ca_3(AsO_3)_2$.

THE ALIMENTARY CANAL OF UNPOISONED LARVAE

The alimentary canal of the sixth-instar larva of the southern armyworm is similar to that of other noctuid larvae. The alimentary canal (pl. 1, *A*) is a straight tube extending from the mouth to the anus and has the usual three primary divisions. The foregut and the hindgut are each about one-third the length of the midgut. They are thin-walled and are lined with a sclerotic intima.

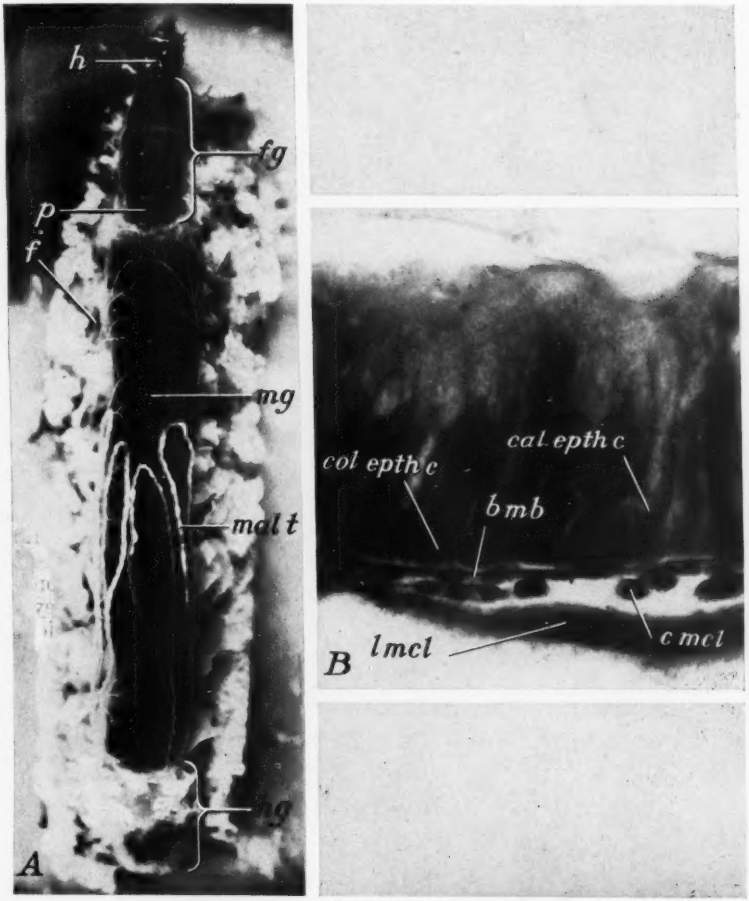
The midgut is a large, undifferentiated tube extending approximately throughout the first six abdominal segments. The canal is normally distended by ingested food materials and in this condition occupies most of the abdominal cavity. The midgut wall (pl. 1, *B*) is made up of an enveloping membrane known as the peritoneum, two layers of muscle fibers, those of the outer layer being longitudinal and those of the inner layer circular, a basement membrane, an epithelial layer, and a thin sheet of detached material closely surrounding the food mass and known as the peritrophic membrane.

The midgut epithelium (Pl. 2, *A*) consists of a single layer of different forms of epithelial cells, of which there are three principal types—columnar, calyciform, and interstitial. The columnar cells are variable in shape and appearance, depending in part on their location and physiological state. They are tall, and often cylindrical, but are usually enlarged distally with slender basal ends. The cell contains an elongate, coarsely granular nucleus. A striated border is present on the free surface of the cell. The slender basal ends of these cells rest on the basement membrane, and the surfaces of the enlarged distal ends are exposed to the gut lumen. The calyciform cells are shaped somewhat like a calyx and fill the spaces between the slender basal parts of the columnar cells, communicating with the free surface by a narrow neck. The cell is often expanded in its middle part. The nucleus lies at the basal end of the cell. Interstitial cells rest on the basement membrane between the bases of the other cells. They are round or elongate in section and contain a large nucleus surrounded by a small amount of strongly basophilic cytoplasm.

The epithelial cells probably function in the secretion of the juices required for digestion, and in the absorption of selected products of the digestion and their transference to the hemolymph. Absorption probably takes place largely, if not entirely, in the midgut.

HISTOPATHOLOGICAL PICTURE OF THE MIDGUT OF POISONED LARVAE

The usual observable effect of the poisons on the gut-wall tissues has been disintegration. The extent of damage has varied according to the kind of poison and the individual larva. The susceptibility of the insect's tissues to the action of the poisons and the quantity and distribution of the poison that reaches the affected areas are probably important factors in the individual variation. The extent of damage in individuals might be directly related to the initial dosage and the time over which the poison has acted, but these factors appear to be inseparable from individual variation unless large numbers of observations are made.



A, Alimentary canal of the sixth-instar southern armyworm exposed by incision along the middorsal line of the body wall, × 3; *B*, longitudinal section from midgut wall of a normal sixth instar, × 650. Abbreviations: *bmb*, basement membrane; *c mcl*, circular muscle fiber; *cal epth c*, calciform epithelial cell; *col epth c*, columnar epithelial cell; *f*, fat body; *fg*, foregut; *h*, head; *hg*, hind-gut; *l mcl*, longitudinal muscle fiber; *mal t*, Malpighian tubules; *mg*, midgut; *p*, proventriculus.

Longitudinal section from midgut wall: *A*, Showing normal epithelial cells, × 1200; *B*, showing injury to epithelial cells in larva poisoned by lead arsenate, × 1200.

ARSENICALS

The mode of action of arsenicals upon protoplasm has received considerable attention from the therapeutic standpoint (*1*, *14*, *15*), but not from the standpoint of insect control. The pathological effects of arsenic compounds on mammals have also been intensively studied, especially in connection with the problems of arsenic therapy (*2*, *6*, *10*). In the mammal a diversity of lesions is produced by different compounds of arsenic, and specificity of action is exhibited toward different species and toward different tissues. The injuries are usually definite structural changes in the cells, leading to disintegration.

Voskresenskaja (*16*) has made a chemical study of the degree and rate of penetration of arsenical compounds through the intestinal walls of several species of insects. Tareev and Nenjukov (*12*) report that sodium arsenite causes complete necrosis of the midgut of *Calliptamus*. Pilat (*11*) found that the gut epithelium of nymphs of *Locusta migratoria* L. had completely disappeared 96 hours after ingestion of this poison, and that the gut of caterpillars of *Aglais urticae* L. was severely damaged. There was no evidence of injury to the gut of larvae of *Pieris brassicae* L. 5½ hours after ingestion of sodium arsenite. Evidence has been presented (*7*, *9*) to show that sodium arsenite and other arsenicals may penetrate the integument of insects and become distributed in the underlying tissues and organs.

Observed effects on the southern armyworm midgut of some arsenicals commonly used in control of different species of insects are described below.

LEAD ARSENATE

By preliminary experiment it was determined that a dose of 0.5 mg. of the lead arsenate is lethal to sixth-instar larvae.

Doses of 0.5 to 1 mg. of the poison were fed to larvae, of which 30 were selected for study. The sandwiches containing the poison were readily consumed, generally within 20 to 30 minutes from the time they were offered. After ingesting the poison the larvae usually became inactive, refusing to feed even on fresh leaf. Within a few hours some of the larvae again became active, but did not feed. Larvae were killed and their gut walls fixed for study 2, 8, 9, 10, and 22 hours after the poison had been ingested.

Damage occurred in midgut walls of larva that had been active immediately before they were prepared for study, as well as of larvae that were already severely affected or apparently dead from the poison. The extent of damage was not clearly related to the time the larvae were held after ingestion of the poison. Apparently any part of the midgut wall may be affected.

The damage to the wall (pl. 2, *B*) is evident from the more or less complete disorganization of the cell structure. The various states of disintegration of epithelial cells are evidenced by the broken-down cytoplasm, vacuolization, merging of the cellular substance of adjacent cells, obliteration of the cell boundaries, disappearance of the striated border, and changes in the nuclei. Fragments of epithelial tissue are sometimes present in the lumen of the canal. Damage to the nuclei is shown in different ways. Part of the nuclear substance may be extruded beyond its original outline in bizarre forms. In some

individuals the nuclear material may be condensed; in others it is broken into particles of irregular form and size. Affected nuclei may stain lightly or not at all, their position being indicated by a faintly stained rim. A few nuclei contained vacuoles. The calyciform cells may be misshapen, or they may stand out distinctly and apparently with little or no damage to either the cell structure or the nucleus, although surrounded by columnar cells in advanced stages of disintegration. The peritrophic membrane is unchanged in appearance except in the most advanced stages of disintegration of the wall; it then may be absent in whole or in part.

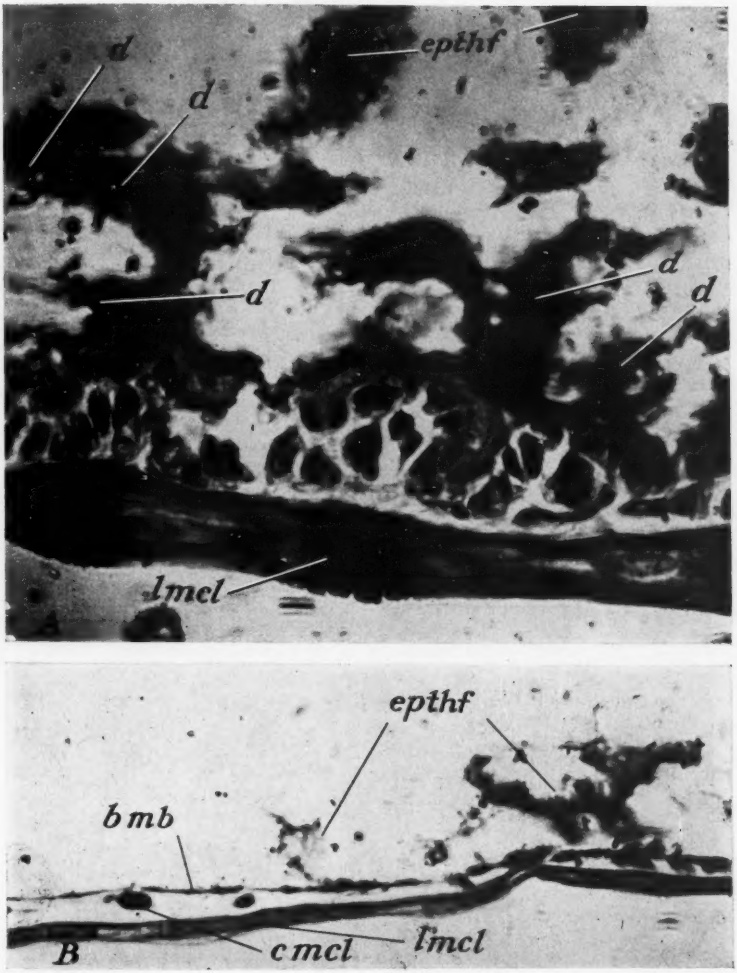
After the death of larvae from lead arsenate poisoning, the epithelium rapidly breaks down and sloughs off (pl. 3, *A*), leaving the basement membrane and muscle layers intact although the muscle fibers are obviously damaged (pls. 3, *B*, and 4, *B*).

A short time after death of the larvae, splotches of black material appear in the wall of the alimentary canal, particularly in the midgut. The material is distinctly visible in both unstained and stained preparations. It may be distributed over the wall as pin-point spots in such a way as to give the wall a sooty appearance. It also occurs in those parts of the Malpighian tubules, labial glands, fat body, and body wall that happen to be in contact with the affected areas of the alimentary canal wall (pl. 4, *A*). The occurrence of the deposit appears to be associated with the presence of the arsenic rather than the lead of the compound, since it did not appear after the larvae had ingested any of three other lead compounds—the acetate, chromate, or nitrate—but it did appear after they had ingested each of five other arsenicals—calcium, sodium, and potassium arsenates, and arsenic trioxide and pentoxide. The substance occurs as a deposit in the tissues of the midgut wall, and particularly in the muscle layers (pl. 4, *B*). The particles are found between the muscle fibers, but there appears to be no actual union with the tissue substance. Scattered deposits occur among the fragments of the disintegrating epithelial tissue (fig. 3, *A*).

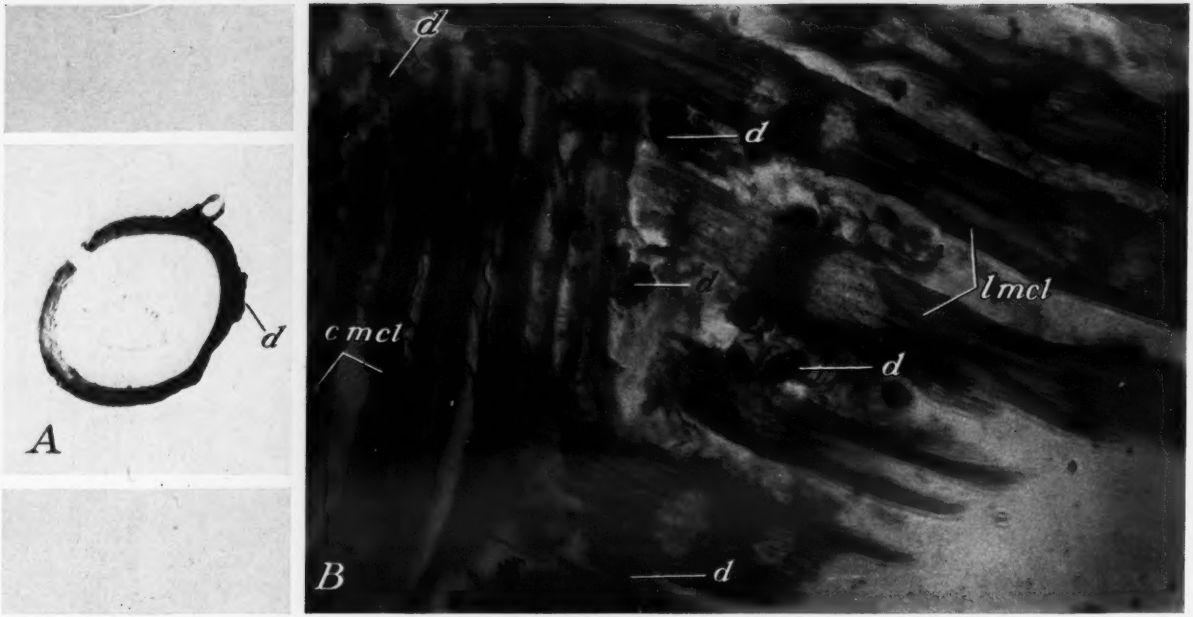
The deposition of arsenicals and other foreign substances in the tissues of animals has frequently been observed (*3*). The deposition and release of such stored substances are accomplished under more or less well-known conditions and may be followed by more or less well-known consequences. Arsenic may form insoluble compounds with proteins in the tissues of mammals, and by reaction with the hydrogen sulfide of the intestinal contents may produce insoluble sulfides. Consideration of the distribution of insecticidal substances in the organs and tissues of insects, and of the conditions affecting their possible storage and release, might lead to fruitful lines of investigation in insect toxicology.

Paris Green

A sandwich containing 0.5 mg. of paris green was fed to each of several larvae, of which six were selected for study. The sandwiches were completely eaten within 20 minutes from the time they were offered. Although supplied with fresh, unpoisoned leaves, the larvae fed no more after ingesting the poison. One larva was killed after 1 hour, at which time it was normally active. The other larvae were killed after 2⅓ to 3½ hours, at which time all showed only feeble signs of life.



Longitudinal section from midgut wall of larva poisoned by lead arsenate: *A*, Showing badly disintegrated and sloughing epithelium and presence of black deposit, × 700; *B*, showing almost complete disappearance of epithelium, only the muscle fibers and basement membrane remaining, × 700. Abbreviations: *bmb*, basement membrane; *c mcl*, circular muscle fiber; *d*, deposit of black material; *epthf*, epithelial fragments; *l mcl*, longitudinal muscle fiber.



A, Transverse section of labial gland of a larva poisoned by arsenic trioxide, showing deposit of black material on side next to the alimentary canal wall in an area containing the black deposit, × 120; *B*, section taken in the plane of the muscle layer of the midgut of a larva poisoned by lead arsenate, showing the particles of black material between the muscle fibers, × 550. Abbreviations: *c mcl*, circular muscle fiber; *d*, deposit of black material; *l mcl*, longitudinal muscle fiber.

The histological sections showed the particles of paris green well distributed in the lumen among the food particles. The epithelial cells showed slight evidence of disintegration similar to that described for lead arsenate, but much less marked. The peritrophic membrane was apparently intact.

Pilat (*11*) found that the midgut epithelium of *Locusta migratoria* nymphs was very little damaged, if at all, 41 hours after ingestion of paris green, but that the epithelium of *Aglais urticae* caterpillars was injured.

Calcium Arsenate

Sandwiches containing 1 mg. of calcium arsenate each were offered several larvae, five of which ate the entire amount. These sandwiches were completely eaten within 1 hour after they were offered. Although supplied with fresh, unpoisoned leaves, the larvae fed no more after ingesting the poison, but regurgitated profusely. One larva was killed 1 hour after ingesting the poison, at which time it was active and apparently healthy. Two were killed 2½ hours after ingesting the poison, when they showed only feeble signs of life. The remaining two were killed 3 hours after ingesting the poison, at which time there was a marked shaking and twitching of the entire body. The tissues of the midgut wall were greatly disorganized, and the epithelial layer appeared as a rather solid mass, occasionally presenting fragments recognizable as cell structures.

Calcium Arsenite

Each of three larvae ate about one-third to one-half of a sandwich containing slightly more than 1 mg. of calcium arsenite. They were obviously very much affected within 2 hours, and after 3 hours they showed only feeble signs of life. The epithelium at this stage was highly disintegrated. The muscle layers were in place, but the fiber structure was broken up. According to Pilat (*11*), the midgut epithelium of *Locusta migratoria* nymphs and of *Aglais urticae* caterpillars is destroyed more or less, depending in part upon the interval between ingestion of the poison and dissection.

Arsenic Trioxide

Two larvae ingested approximately 1 mg. of arsenic trioxide each within half an hour. One larva was killed 2 and the other 3 hours after ingesting the poison, at which times they showed only feeble signs of life. The midgut epithelium of these larvae showed only slight, if any, injury due to the poison. A third larva ate an entire sandwich containing 5 mg. of the poison, ceased feeding immediately thereafter, and regurgitated profusely. It moved with difficulty after 5 hours. It was killed after 9 hours, at which time respiratory movements were the only signs of life. The entire midgut wall was severely damaged, as shown by merging of the cytoplasm and general disorganization of all the tissues of the epithelium.

O'Kane and Glover (*9*) demonstrated the passage of arsenic trioxide through the integument of *Periplaneta americana* (L.), and its distribution in the tissues of the internal organs.

BARIUM FLUOSILICATE

Sandwiches containing from 0.2 to 1 mg. of barium fluosilicate were readily ingested within about one-half hour. Eight larvae had eaten sandwiches containing 0.5 to 1 mg. of poison, and three others ate sandwiches containing 0.2 to 0.33 mg. After ingesting the poison one larva ate an unpoisoned sandwich and then became inactive. All others refused to eat, and all were inactive. Poison effects were shown by sluggishness, limpness, abnormal jerking movements with or without stimulation, and occasional regurgitation.

Larvae were killed and prepared for study 1, 3, and 5 hours after ingestion of the poison, and also when they appeared to be near death, which was usually within 8 or 9 hours.

In the histological preparations it was impossible to detect changes in the epithelium that could be attributed with certainty to the action of the barium fluosilicate. The individual cells showed little or no evidence of injury, and certainly no such disintegration as occurred with arsenicals. The peritrophic membrane was intact.

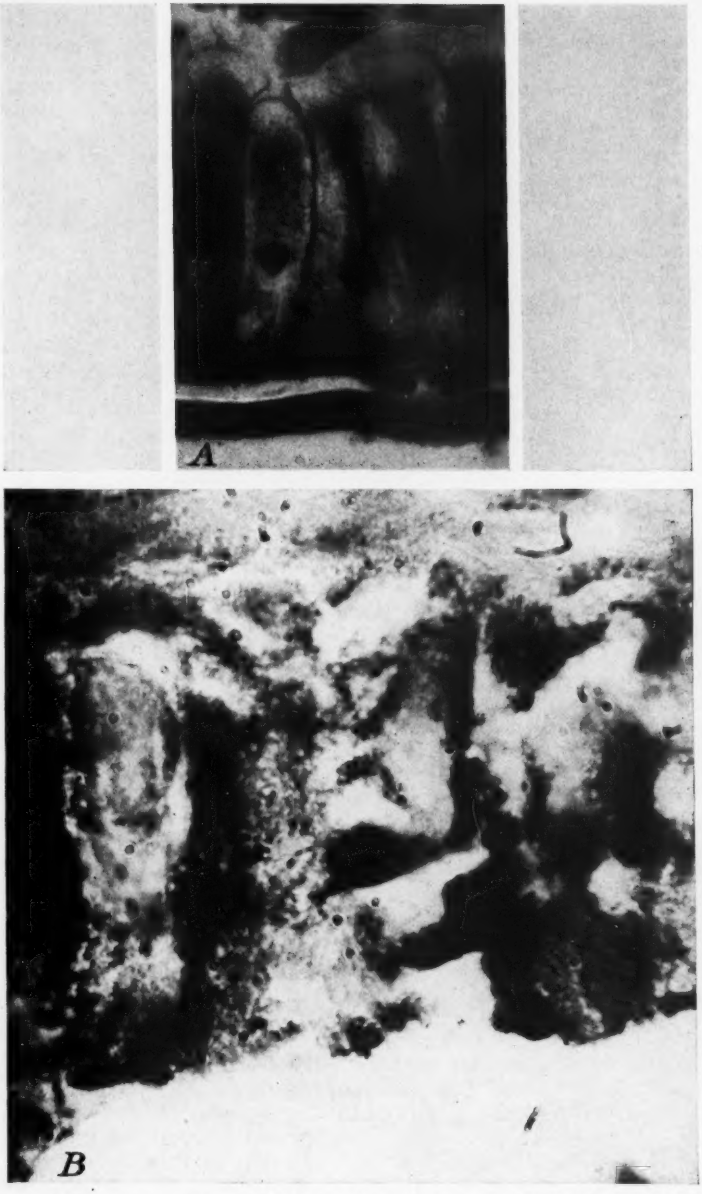
SODIUM FLUORIDE

Sodium fluoride in doses of 2 to 5 mg. was fed to the larvae. This quantity is lethal, death occurring within 3 to 12 hours. Two sandwiches containing 5 mg. of the poison were completely eaten within 1 hour, and several others were partially eaten within 2½ hours. After ingesting the poison, the larvae occasionally regurgitated and showed abnormal movements such as sudden rearing of the head and thorax, twisting from side to side, and turning ventral side up. Affected larvae often were unable to crawl. They were inactive most of the time and refused to feed on fresh leaf. After a few hours they became limp.

Larvae were killed and their gut walls fixed for study 5 to 10 hours after ingesting the poison, at which time they usually showed only feeble signs of life, such as faint respiratory movements and slight activity of the appendages on stimulation with a needle.

Damage (pl. 5, *A*) to the midgut wall was shown in disintegration of the substance of the cytoplasm and nuclei of the epithelial cells. The cytoplasm of the columnar epithelial cells often appeared disrupted, usually at the distal or lumen end, giving this part of the cells a ragged appearance. Damage to the nuclei of the calyciform as well as to those of the columnar epithelial cells is shown in part in a fragmented and misshapen condition of the nucleus. Frequently the nuclei appear as solid-staining, apparently shrunken bodies, rather than as normal granular bodies. Occasionally the nuclei of the columnar cells had almost disappeared, being located merely by a stained ring representing the outline of the nucleus, and by scattered granules inside the ring. The calyciform cells present the usual outline and appearance of these cells. The peritrophic membrane was unchanged in appearance.

Pilat (*11*) found that the epithelium of *Locusta migratoria* nymphs and *Aglais urticae* caterpillars is damaged by sodium fluoride. Hockenyos (*5*) found that sodium fluoride can be absorbed in lethal amounts directly through the body integument of *Periplaneta americana* and *Blatta orientalis* L.



Longitudinal sections from midgut wall of poisoned larva: *A*, Larva poisoned by sodium fluoride, × 620; *B*, larva poisoned by sodium fluoaluminate, showing injury to epithelial cells, × 1400.

SODIUM FLUOALUMINATE

Five larvae each readily ingested a sandwich containing 1 mg. of synthetic sodium fluoaluminate within 30 minutes. They were then quiescent for about an hour, after which they again became active without immediately showing symptoms of poisoning.

Two larvae were killed and their gut walls prepared for study 9 hours after they had ingested the poison, at which time one was unusually sluggish and the other jerked its body in an abnormal manner. Three larvae were killed 24 hours after ingesting the poison, at which time they were active and showed no such abnormal behavior.

The midgut walls of all these larvae were greatly disintegrated, and there was no clear distinction in effects between the larvae killed 9 hours and those killed 24 hours after ingesting the poison. The cross striations of the muscle fibers were faint or obliterated. Damage to the epithelium (pl. 5, *B*) was seen from the disorganization of the epithelial cells, merging of the cytoplasm of adjacent cells, and changes in the nuclei. The most frequently observed evidence of damage to the nuclei was fragmentation. In the same microscopic field with fragmented nuclei would often be others more or less shrunken and deeply stained, and still others apparently in the process of fading out. Exfoliation of the entire epithelial layer from the subjacent basement membrane occurred over extensive areas.

PHENOTHIAZINE

One larva was offered a sweetpotato-leaf sandwich containing approximately 1 mg. of phenothiazine. It immediately ate small portions from the edge of the sandwich, and then fed no more either on the sandwich or on fresh leaf. Within 24 hours it had become flabby and apparently lifeless except for feeble movements when touched with a needle. There was no obvious change in its condition after 60 hours. At this time a lighted electric bulb was brought near the larva, causing movements of the thoracic legs, followed by a more vigorous movement in which the fore part of the body was thrown backwards until it touched the hindermost part of the body. When the bulb was removed, the larva immediately resumed its apparently lifeless and flabby appearance.

The larva was then killed. Microscopical examination revealed no structural differences in any of the tissue elements of the midgut that could with certainty be attributed to an action of the phenothiazine. Even the delicate striated border was remarkably well preserved.

ROTENONE

Five southern armyworm larvae each ingested a sweetpotato-leaf sandwich containing 5 mg. of fine, chemically pure rotenone. The larvae were then placed on a living turnip plant, upon which they fed and reacted normally. From 75 to 84 hours later there were no changes in the microscopic appearance of the epithelial cells or other tissues of the midgut that could with certainty be attributed to rotenone.

Rotenone dissolved in olive oil may cause severe hyperemia of the entire digestive tract of white rats (*8*). The effects of rotenone upon fish (*4*) consist primarily in the destruction of the gill epithelium, which makes the inspiration of oxygen impossible.

Rotenone varies remarkably in its toxic action on different species of insects. Ingested rotenone apparently has no toxic action on the southern armyworm, and most of it passes through the insect unchanged (*18*). Cube, in which rotenone is the principal insecticidal ingredient, also is apparently ineffective as a stomach poison against the southern armyworm (*17, pp. 62–63*).

In the present work silkworm larvae died within about 2 hours from the effects of ingesting minute quantities of rotenone, whereas southern armyworm larvae readily ingested 5 to 10 mg. of rotenone in sandwiches without showing any ill effects.

SUMMARY

Histopathological effects of certain insecticides on the midgut wall of the sixth-instar southern armyworm (*Prodenia eridania* (Cram.)) were studied. Lethal or large doses of poisons were fed by the sandwich method, and after sufficiently long intervals of time the larvae were killed and the tissues prepared by histological methods. Similar preparations of larvae that had not ingested poisons were used for comparison.

The different insecticides showed differences in action ranging from no apparent effects to well-marked damage to the midgut wall. The ingestion of arsenicals (lead arsenate, paris green, calcium arsenate, calcium arsenite, and arsenic trioxide) was followed by disintegration of the midgut epithelial cells and damage to the midgut muscle fibers. Disintegration of the substance of the cytoplasm and nuclei of the epithelial cells followed the ingestion of sodium fluoride. The epithelial cells of larvae that had ingested sodium fluoaluminate were greatly disintegrated, and the cross striations of the muscle fibers were faint or obliterated. The ingestion of barium fluosilicate, phenothiazine, and rotenone was followed by no changes in the epithelium or muscle fibers that could be attributed with certainty to these substances.

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THE INFECTION CAPABILITIES OF HOP DOWNY MILDEW[1]

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INTRODUCTION

Downy mildew, *Pseudoperonospora humuli* (Miyabe and Tak.) Wils, was first observed in commercial hopyards in Oregon in 1930. Following a survey to determine the incidence of infection, an investigation of the life history and infection capabilities of the causal organism was inaugurated. The primary objectives of the investigation were to determine the host range of the fungus; to discover any evidence of resistance to the fungus among botanical species, horticultural varieties, or strains of hops developed by means of selection or hybridization; and by attempting to infect all available known hosts of other species of the genus *Pseudoperonospora*, to confirm or disprove the opinion [2] that physiological races of the fungus may exist.

MATERIALS AND METHODS

In a previous paper the writer [3] discussed in some detail materials and methods employed and results obtained early in the course of this investigation.

In addition to hop seedlings, the leaves of hop plants grown from cuttings were employed. Both cotyledons and leaves of seedlings, as well as the leaves of more mature plants of other host genera, were used. In some instances excised cotyledons and leaves were incubated after inoculation in Petri dish moist chambers or floated in covered Petri dishes of nutrient or sugar solutions.

The methods of applying the inoculum varied also. In addition to the use of camel's-hair brushes for the transfer of inoculum, water suspensions of zoosporangia were atomized onto the plant parts or placed in localized areas by means of a medicine dropper.

HOST RANGE

An extensive series of inoculations with the zoosporangia of the hop downy mildew fungus was begun in 1931 and continued each year through 1937.

The original inoculum was obtained from naturally infected hop plants in Oregon yards.

Only positive results are recorded here. Optimum environmental conditions were not subject to accurate control, and to this circum-

[1] Received for publication June 17, 1940. Cooperative investigation by the Division of Drug and Related Plants, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon Agricultural Experiment Station. Technical Paper No. 295, Oregon Agricultural Experiment Station.

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stance some of the negative results obtained doubtless can, in part at least, be attributed.

Results are herein recorded as positive when the organism invading the infected host developed zoosporangia, whether or not accompanied by distinctly visible lesions on the inoculated parts not found on similar uninoculated parts.

Source of inoculum	*Host infected*
Humulus lupulus L.	
Variety unknown	*Humulus lupulus* L.: Early Clusters. Early Green. East Kent Golding. Fuggles. Late Clusters.
Early Clusters	*Celtis mississippiensis* Bosc. *C. occidentalis* L. *C. sinensis* Pers. *C. tournefortii* Lam. *Humulus lupulus:* Early Clusters. Fuggles. Late Clusters. Red Vine.
Fuggles	*Humulus lupulus:* Early Clusters. Fuggles. Late Clusters. Red Vine.
Late Clusters	*Cannabis sativa* L.: Bologna. Kymington. Palmate. Pinnatifid Simple Leaf. Simple Leaf. Tochigi. *Celtis mississippiensis.* *C. occidentalis.* *C. sinensis.* *C. tournefortii.* *Humulus japonicus* Sieb. and Zucc. *H. japonicus* var. *variegatus* Hort. *H. lupulus:* "Bohemian." Early Clusters. East Kent Golding. Fuggles. Late Clusters. Red Vine. *H. lupulus* var. *neo-mexicanus* Nels. and Ckll. *Urtica lyallii* Wats.
Red Vine	*Humulus lupulus:* Fuggles. Red Vine.
Humulus lupulus l. var. *neo-mexicanus* Nels. and Ckll.	*Humulus lupulus:* Fuggles. Late Clusters. Red Vine. *Humulus lupulus* var. *neo-mexicanus.* *Urtica lyallii.*
Urtica lyallii Wats.	*Humulus lupulus:* Fuggles. Late Clusters. *Urtica lyallii.*

It is of interest, in passing, to note that all attempts to infect available hosts of *Pseudoperonospora cubensis* (B. and C.) Rostowzew, *P. erodii* (Fckl.) Wils., and *P. portoricensis* (Lamkey) Hoerner [4] were unsuccessful. Host material of *P. elatostemae* (Togashi and Onuma) Hoerner [4] was not available.

RESISTANCE

Of the botanical species of hops inoculated, *Humulus japonicus* and *H. japonicus* var. *variegatus* showed some evidence of resistance. This fact is of doubtful economic importance, however, since both species are annuals and may not, in view of present knowledge,[5] be suitable for hybridization with the perennial species and varieties of *H. lupulus*, from which many of the hops of commerce are derived.

In addition to the inoculation work recorded above, field observations were made and additional inoculation work was carried on with a number of standard varieties, seedlings, and strains of hops developed by selection or hybridization at the Oregon Agricultural Experiment Station in cooperation with the Bureau of Plant Industry, in search for possible immunity to downy mildew. No immunity was found. Apparent differences in susceptibility which have appeared in certain lines have not maintained themselves consistently in subsequent tests. The evidence leads to the conclusion that individual plants may escape infection, for various reasons, for several years, although they are not genetically resistant.

The development of zoosporangia resulting from inoculation was relatively sparse on all other hosts as compared with that on *Humulus* spp. Large hypersensitive areas appeared consistently on the cotyledons of *Celtis occidentalis* and on both cotyledons and leaves of *C. mississippiensis*, *C. sinensis*, and *C. tournefortii*. Similar evidence of "subinfection" was a common occurrence also on the leaves of *Urtica lyallii*.

The hypersensitive areas referred to were definitely necrotic but indefinite in outline (fig. 1). The surface, in time, usually became depressed in relation to the surrounding normal tissue. Affected tissue became discolored through varying shades of yellow, brown, or black. Oftentimes no zoosporangia were produced in the necrotic area; when produced, they were noticeably fewer than those produced on infected plant parts not developing the hypersensitive areas.

These phenomena indicate that the different host species provided different physiological environments for the invading organism.

In view of the fact that all species tested of the genera *Cannabis*, *Celtis*, *Humulus*, and *Urtica*, which are hosts respectively of *Pseudoperonospora cannabina* (Otth) Hoerner, *P. celtidis* (Waite) Wils., *P. humuli* (Miyabe and Tak.) Wils., and *P. urticae* (Lib. ex Berk.) Salm. and Ware, became infected with *P. humuli*, it would seem not outside the range of possibility that they may, after all, prove to be only different physiological races of one of the species of the genus *Pseudoperonospora*.

[4] HOERNER, G. R. A NOMENCLATORIAL NOTE ON PSEUDOPERONOSPORA. Wash. Acad. Sci. Jour. 30: 133-134. 1940.

[5] WING, Ojvindj. III. THE POLLINATION AND FERTILIZATION PROCESSES IN HUMULUS LUPULUS L. AND H. JAPONICUS SIEB. AND ZUCC. *In* Schmidt, Johs., Investigations on Hops (*Humulus lupulus*, L.). Carlsberg Lab. Compt. Rend. des Trav. 11: 1–44, illus. 1914.

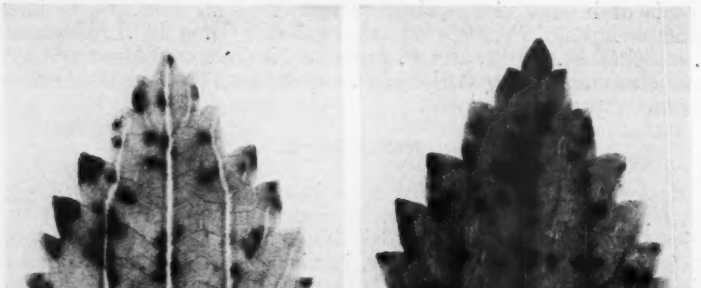

FIGURE 1.—Lower surface (*A*) and upper surface (*B*) of nettle leaf (*Urtica lyallii*) infected with hop downy mildew (*Pseudoperonospora humuli*), showing hypersensitive areas.

SUMMARY

The host range of the hop downy mildew fungus was found to be considerably more extensive than had previously been reported. All hosts, however, were members of the nettle family.

Of the botanical species of hops inoculated, *Humulus japonicus* and *H. japonicus* var. *variegatus* showed some evidence of resistance. Evidence of resistance on the part of horticultural varieties or strains of hops developed by means of selection or hybridization was inconclusive. Further study to confirm preliminary indications is necessary before definite conclusions would be justified.

In view of the infection by the hop downy mildew of species of *Cannabis*, *Celtis*, and *Urtica*, hosts respectively of *Pseudoperonospora cannabina*, *P. celtidis*, and *P. urticae*, it is suggested that the forms of *Pseudoperonospora* described under these names, along with *P. humuli*, may eventually prove to be physiological races of a single species.

CHEMICAL AND PHYSICAL PROPERTIES OF SOILS AND OF THEIR COLLOIDS DEVELOPED FROM GRANITIC MATERIALS IN THE MOJAVE DESERT [1]

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INTRODUCTION

The opinion has been prevalent that desert soils for the most part are composed of sandy and gravelly materials which are loose and porous in character except for local areas of lime and gypsum hardpans. Lapham (*22*) [2] objected to this view and suggested that this concept developed through theoretical considerations and from the fact that the early studies of Hilgard and others were largely directed to areas of slightly weathered alluvial soils which were well situated with reference to water supply for irrigation. Thus Hilgard (*16*) stated that in the arid regions "the formation of colloidal clay is very much diminished, so that most soils formed under arid conditions are of a sandy or pulverulent type."

More recent studies of soils in arid regions indicate that the formation of soils with considerable clay content is common in areas of limited rainfall, both in the northern and southern deserts. In many of the recent soil surveys of southern Arizona and California, typical mature desert soils are described in which the subsoil contains a relatively large amount of clay (*21*, *31*).

Nikiforoff (*26*), in a morphological study of the soils of the Mojave Desert, found that one of the principal zonal characteristics of southern desert soils is the presence of a reddish-brown clay layer, or "claypan," in the lower part of the solum. In areas of higher rainfall the cause of claypan formation is assigned by Brown, Rice, and Byers (*6*) to the flocculation and filtering out of the eluviated colloid. Bray (*3*) concluded that in certain Illinois soils a claypan is formed by vertical transfer of clay without much alteration in its composition. Nikiforoff (*26*) did not find any evidence in the field study of the Mojave Desert soils to indicate an illuvial origin of the claypan. He suggested that the clay layer is formed in place by the hydrolytic decomposition of the primary minerals. It seemed desirable to make a laboratory study of the soils of this area in order to obtain further evidence bearing on this hypothesis and to add to our meager knowledge of the chemical composition of desert soils.

DESCRIPTION OF THE SOILS

The soils were collected by C. C. Nikiforoff, of the Division of Soil Survey, and the junior author. Special care was taken to select soils with a more or less distinct claypan; one soil, however, without such a horizon (profile F) was also sampled as a representative of the

[1] Received for publication August 3, 1940.

[2] Italic numbers in parentheses refer to Literature Cited, p. 350.

common but apparently underdeveloped soils of the alluvial fans in the desert.

The samples were collected in the southwestern part of the Mojave Desert. Five profiles were selected within a few miles of Mojave, in Kern County, and one profile (B) in San Bernardino County, both in California.

The climate of the Mojave Desert is arid and hot. It is of the so-called Arizona type that is characterized by a distinct winter concentration of rainfall. The mean annual rainfall is slightly less than 5 inches, and more than three-fourths of it falls during the winter. The mean annual temperature is about 65° F.

The winter rains are gentle and practically all the rainfall is absorbed by the soil, which usually becomes moist during this period to a depth ranging from about 2 to more than 5 feet, depending upon the annual variation of the seasonal rains. This moisture permits the seeds of annual plants to germinate and supports the short but sometimes very spectacular spring outburst of desert flora. The return of the usual summer aridity and heat of the desert quickly terminates the vegetative period. The rains during the summer are rare and usually fall as heavy showers of short duration. Most of this water is lost through run-off; it seldom penetrates the soil more than 1 inch, and the soil dries up almost as fast as the rain passes. Only the most drought-resistant perennial plants survive throughout the year; the most typical of these are the creosotebush (*Larrea divaricata* Cav.), certain sages, the Joshua-tree (*Yucca brevifolia* Englem.), and several varieties of cactus.

As the area in which the soil samples used in this investigation were collected has not been covered by a detailed survey, the soils have not as yet been correlated. The soils developed from a residual granite material (profiles A, B, and C) are tentatively grouped into the Muroc series. This name, however, is subject to possible change when the final correlation is made. The soil represented by profile E is very similar to the soils of the Mojave series. No names were suggested for the soils represented by profiles D and F.

A short description of each profile as furnished by the collectors follows:

PROFILE A

Location.—Mojave desert, 7.75 miles east of Rosamond, along the road to Muroc and about 2.25 miles north of highway. Elevation about 2,900 feet. A gentle slope to the south from the base of a low rocky ridge. Surface drainage is good.

Vegetation.—Widely spaced creosotebushes and other desert shrubs; a few single Joshua-trees. Much of the surface between the shrubs is bare and covered with a thin coat of angular grit.

Parent material—Very gritty and derived from granite. Rotten rock generally occurs at a depth ranging from a few inches to more than 2 feet from the surface. Numerous outcrops of bedrock are scattered all over the area.

Description of the profile:

1 (?). 0–2 inches. Probably overwash. Loose gritty loam of a medium brownish-gray color.

2 (B_1). 2–5 inches. A very compact gritty clay of a bright dark reddish-brown color; sticky when wet, hard and massive when dry.

3 (B_2). 5–11 inches. Practically the same texture, color, and consistence as the overlying horizon but differs from the latter in structure. It breaks into well-defined roughly prismatic clods. The surfaces of these clods are much darker than the interior.
4 (B_3). 11–14 inches. Gritty clay of a less brilliant and somewhat lighter reddish-brown color. It breaks on drying into coarse irregular lumps.
5 (C). 14–34 inches. Rotten granitic rock. To about 40 inches from the surface this material has a pinkish-brown to rusty-brown color. Further down the pink and brown tints disappear and the disintegrated rock retains the light-gray color of the original solid granite.

The free carbonates appear at the depth of 12 inches from the surface. In B_3 subhorizon, a weak pseudomycelium is formed. In the disintegrated rock, carbonates form numerous anastomosing mostly horizontal streaks, ranging in thickness from a mere film to more than 1 inch.

PROFILE B

Location.—Mojave desert, 7.4 miles north of Kramer corner (crossroad 35.5 miles east of Mojave). About 300 feet west of highway. Elevation about 3,000 feet. Low, very gently sloping granitic ridge. Surface drainage is good.

Vegetation.—Sparsely scattered desert shrubs with a practically bare surface between them.

Parent material.—Residual from granitic rock.

Description of the profile:

1 (?). 0–7 inches. Probably overwash. Friable gritty loam of a dull medium- to light-brown color. Its thickness at different places ranges from less than 5 inches to about 2 feet.
2 (B_1). 7–10 inches. Compact gritty clay loam of a bright but rather dark reddish-brown color. It breaks on drying into roughly prismatic clods.
3 (B_2). 10–14 inches. The same color, texture, and consistence as B_1, but it does not crack as much. It is highly calcareous and differs in this respect from B_1, which is practically free from carbonates. The carbonates form numerous pure-white segregations, ranging in diameter from a small fraction to about 1 inch.
4 (B_3). 14–24 inches. Strongly weathered gritty granitic material impregnated with abundant streaks formed by carbonates.

PROFILE C

Location.—10.9 miles east of Mojave along the highway to Barstow and 1 mile north of the highway. Elevation about 2,800 feet. Undulating to gently rolling topography of a wide and low granitic ridge. Surface drainage is good.

Vegetation.—Sparse creosotebushes and other desert shrubs. Practically bare surface between the bushes.

Parent material.—Residual from granite. Rotten rock occurs at a depth ranging from less than 1 to more than 2 feet.

Description of the profile:

1 (?). 0–6 inches. Loose, structureless, gritty sandy loam of a light grayish-brown color.
2 (A_2). 6–10 inches. Somewhat heavier in texture, slightly more reddish in color and considerably more compact than 1.
3 (B). 10–17 inches. Gritty clay loam of a medium-brown to reddish-brown color and of a compact but crumbly consistence. Breaks on drying into poorly defined but roughly prismatic clods.
4 (C_1). 17–26 inches. Crumbly, disintegrated granite of yellowish- to rusty-brown color, with numerous red spots and films in cracks.
5 (C_2). 36–48 inches. Light-gray disintegrated granite, considerably less crumbly than C_1.

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Carbonates appear at a depth of about 9 or 10 inches from the surface.

PROFILE D

Location.—3.55 miles north of Mojave along the highway to Bishop. Elevation about 3,000 feet. Broad alluvial fan, very gently sloping eastward from the base of Tehachapi Mountains toward the playa.

Vegetation.—Creosotebushes, with a few scattered Joshua-trees. Practically bare surface between the shrubs.

Parent material.—A gritty indistinctly stratified water-laid deposit.

Description of the profile:

1 (?). 0–12 inches. Probably mechanical overwash rather than a genetic horizon. Structureless gritty loamy sand to gritty sand of a light-brown color and of a friable consistence. It is covered by a thin somewhat compacted but fragile crust underlain by a porous (vesicular) material of very light-gray color extending from 1 to 2 inches from the surface.

2 (B). 12–24 inches. Gritty sandy clay loam of a bright-brown or reddish-brown color and of a compact consistence. It is sticky when wet and breaks into irregular, roughly prismatic clods when dry.

3 (C_1). 24–36 inches. Brittle, weakly carbonate cemented hardpan, composed of gritty sandy clay loam with a small admixture of gravel.

4 (C_2). 36+ inches. Crumbly to friable gritty sand of light grayish-brown color. Content of gravel increases gradually in deeper strata, but stratification is rather indistinct. Segregation of carbonates in pockets and streaks produces a slight local cementation.

Carbonates appear at a depth of about 27 inches from the surface. At a depth of 50 inches and deeper, the content of carbonates increases and they segregate in crumbly, more or less horizontal streaks ranging in thickness from a mere film to more than 1 inch.

PROFILE E

Location.—3 miles east of Mojave along the highway to Barstow. Elevation about 2,700 feet. Broad flat at the base of Tehachapi Mountains. The general surface is nearly level, marked by a slightly developed microrelief. Surface drainage is fair.

Vegetation.—Sagebrush and other desert shrubs, with very scant growth of annuals between the shrubs. Areas of practically bare surface are numerous.

Parent material.—Presumably stratified old water-laid deposits (basin filling) of undetermined thickness.

Description of the profile:

1 (?). 0–5 inches. Probably a recent mechanical overwash, loose, structureless, gritty loam of a medium grayish-brown color.

2 (B_1). 5–10 inches. Gritty clay loam of a reddish-brown color and very compact consistence. Breaks into coarse angular clods when dry; massive and very sticky when wet.

3 (B_2). 10–20 inches. Gritty clay loam of a much brighter and somewhat darker reddish-brown color and of a somewhat heavier texture than in the B_1. It has a very compact consistence and breaks into well-defined, roughly prismatic clods on drying.

4 (B_3). 20–34 inches. Gritty clay loam of a somewhat lighter and duller color than that of B_1 and B_2; more or less massive, very compact, and moderately cemented by carbonates. The carbonates form a very fine moldlike pseudomycelium that thoroughly penetrates the subhorizon. A small admixture of gravel is present.

5 (C). 34+ inches. Loose coarse sand, grit, and gravel mixed indiscriminately. General color is light grayish brown.

PROFILE F

Location.—4 miles north of Mojave along the road to Bishop and 1 mile east of the highway. Elevation about 2,800 feet. Lower part of a broad alluvial fan. An exceedingly gentle general slope eastward. Surface drainage is good.

Vegetation.—Widely spaced growth of creosotebushes; an occasional Joshua-tree. Between the creosotebushes are interspread *Grayia spinosa* (Hook.) Moq., *Cassia armata* Wats., *Franseria dumosa* Gray, and several other desert shrubs, *Eriogonum inflatum* Torr., *Oxytheca perfoliata* Torr. and Gray, *Gilia*, etc.

Parent material.—An indistinctly stratified fan alluvium several hundred feet thick.

Description of the profile:

1 0–3 inches. Very porous (vesicular) and friable sand of very light-gray color. Its surface is compacted into a thin crumbly crust covered by a coat of granitic grit.

2 3–16 inches. A structureless friable sand to loamy sand with a small admixture of granitic grit; very light brown in color.

3 16–48 inches. Similar to the above horizon except for a somewhat finer texture near the base and a moderate content of carbonates, which are disseminated throughout the material without any distinct efflorescence.

4 48+ inches. A structureless sandy loam of a dull-brown color thoroughly mottled with a rich pseudomycelium of carbonates. It is rather mellow in a moist condition but bakes on drying into a massive and slightly cemented pan.

METHODS OF EXAMINATION

The methods used in determining the various constituents and properties of the samples of soils and the colloids extracted from them were essentially those in general use by the Division of Soil Chemistry and Physics. They have been described in detail in the publications cited below.

All of the soils were air-dried before making the laboratory determinations. The mechanical analyses were made by the pipette method described by Olmstead, Alexander, and Middleton (*27*), except for the added changes mentioned by Knight (*20*). As this method requires the removal of organic matter, the approximate percentages obtainable by this procedure are also recorded. The hydrogen electrode described by Bailey (*2*) was used in determining the pH value of the soil. The soluble salts were determined by the bridge method described by Davis (*8*). The ratio of water to soil was 10 to 1.

The chemical analyses of both the soil and colloid were made according to the procedure described by Hillebrand (*17*) and as outlined by Robinson (*29*). Replaceable bases and base-exchange capacity were determined at pH 7.0, normal ammonium acetate being used as the replacing agent.

The procedure used for extracting the colloids from the soils has been described by Brown and Byers (*4*). No dispersion agent was used. The soil was dispersed in 3 gallons of distilled water, by means of a mechanical stirrer described by Holmes and Edgington (*18*). The suspension was decanted into 7 gallons of water and centrifuged at the rate of 17 seconds per liter at a speed of 16,500 revolutions per minute (bowl diameter, 4 inches). The colloid, still in suspension, was collected on a Pasteur-Chamberland filter, and the filtrate was used again to disperse the sediment removed from the centrifuge bowl. The process was repeated two to four times, until an adequate amount

of colloid was obtained. Very few of the colloid particles exceeded 0.3μ in diameter; most of them were much smaller.

The data are presented in separate tables. Certain derived data have been segregated. In these, only the oxides of magnesium, calcium, potassium, and sodium are referred to as bases. In calculating certain derived data, the percentage of each constituent is divided by its formula weight. These quotients are compared in the ratios.

ANALYTICAL RESULTS

The mechanical composition of the six profiles is presented in table 1. The analyses in this and in other tables are divided into two parts: (1) Soils derived from granite and (2) soils derived from alluvial fan materials. Each of these divisions is arranged in the order of the profile development of the claypan in them. In these mechanical analyses the usual procedure of using only the portion consisting of particles less than 2 mm. in diameter is adopted, as this is the portion usually defined as soil. With these and other data, some of the possible causes of claypan formation in desert soils will be explored.

TABLE 1.—*Mechanical analyses of Mojave Desert soils* [1]

Profile and sample No.	Horizon	Depth	Fine gravel (2–1 mm.)	Coarse sand (1–0.5 mm.)	Medium sand (0.5–0.25 mm.)	Fine sand (0.25–0.1 mm.)	Very fine sand (0.1–0.05 mm.)	Silt (0.05–0.002 mm.)	Clay (0.002–0 mm.)	Organic matter from H_2O_2	Less than 0.005 mm.
DERIVED FROM GRANITE											
Profile A:		*Inches*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
C3419	1	0–2	21.6	16.8	6.2	10.2	10.7	24.8	9.0	0.6	13.3
C3420	2	2–5	16.7	15.1	5.3	9.0	7.3	10.2	35.9	.4	38.0
C3421	3	5–11	19.0	12.0	3.7	7.0	9.7	13.6	34.5	.3	36.4
C3422	4	11–14	11.7	9.9	4.3	9.8	14.7	19.6	29.5	.3	32.0
C3423	(2)	14–34							14.0		
C3424	(2)	44–50									
Profile B:											
C3410	1	0–7	16.2	22.4	13.2	16.9	6.8	9.5	14.6	.2	17.0
C3411	2	7–10	14.3	19.2	11.4	15.1	6.0	9.6	24.0	.2	26.1
C3412	3	10–14	15.8	18.9	9.6	12.6	5.1	10.3	27.3	.2	29.9
C3413	4	14–24	41.4	22.2	6.0	5.7	2.0	7.2	15.0	.4	17.3
Profile C:											
C3401	1	0–6	15.4	14.9	9.1	21.2	11.5	14.0	13.7	.1	16.0
C3402	2	6–10	9.7	10.6	8.5	24.9	14.8	15.3	16.1	.0	18.8
C3403	3	10–17	19.4	13.7	7.6	16.2	8.9	13.6	20.3	.1	22.8
C3404	4	17–26	53.6	22.4	4.3	4.2	1.5	6.7	7.2	.0	9.1
DERIVED FROM ALLUVIAL FAN MATERIALS											
Profile D:											
C3395	1	0–3	8.7	22.2	14.3	23.7	10.9	10.9	8.6	0.4	12.4
C3396	2	3–12	9.2	23.8	16.5	27.7	9.0	4.2	9.5	.0	10.8
C3397	3	12–24	8.2	15.6	11.3	20.0	10.7	10.3	23.8	.0	26.1
C3398	4	24–30	11.0	16.6	11.6	22.0	8.8	9.2	20.7	.0	22.5
C3399	5	30–36	25.4	19.4	10.5	16.7	6.2	7.6	13.9	.1	16.6
C3400	6	36–50	32.5	28.7	12.9	11.4	2.1	3.4	8.3	.6	8.8
Profile E:											
C3414	1	0–5	12.0	16.8	12.1	24.1	12.1	8.8	13.6	.3	16.4
C3415	2	5–10	7.3	12.2	10.1	23.9	14.8	9.1	22.5	.0	25.1
C3416	3	10–20	12.0	17.3	10.8	17.7	7.7	7.2	26.7	.4	28.9
C3417	4	20–34	10.7	19.9	14.2	21.3	5.6	6.5	21.2	.3	23.0
C3418	5	34–44	22.2	25.5	15.0	18.3	4.3	4.7	9.9	.0	10.9
Profile F:											
C3879	1	0–3	13.0	25.2	16.8	24.8	9.5	6.2	4.1	.2	4.6
C3880	2	3–6	6.8	19.2	16.3	30.9	10.6	9.3	6.7	.0	7.8
C3881	3	6–16	6.3	19.0	16.1	31.2	12.1	6.7	8.5	.0	9.8
C3882	4	16–24	12.5	24.3	15.6	26.1	8.7	5.0	7.7	.0	8.8
C3883	5	24–40	5.4	21.0	17.7	34.5	10.8	3.6	6.9	.0	7.1
C3884	6	40–48	2.3	13.5	16.2	16.9	35.9	6.2	8.9	.0	9.6
C3885	7	48–60	2.3	5.4	7.2	25.8	19.0	25.6	14.5	.0	20.3

[1] Analyses by T. M. Shaw, E. F. Miles, and A. E. Yelmgren.
[2] Broken rock.

The first five of the profiles have a marked accumulation of clay in the part commonly defined as the B horizon. The last profile does not. The surface horizon of each profile may be overwash, as indicated in the descriptions of the profiles.

In profile A, horizon 2 has nearly 36 percent clay, and horizon 3 has only a little less. Horizon 4 rests on the broken parent rock and contains still less clay. Profiles B and C are similarly developed, though the percentage of clay in the subsoil horizons is not so high as in A.

The last three profiles in table 1 are developed on alluvial fan materials. The character of these profiles is necessarily somewhat different from that of the residual soils. It is evident that their texture may be modified by the quantity, the kind, and the size of rock fragments poured out and deposited on the alluvial fans by the intermittent torrential floods issuing from the often dry beds of mountain streams. The upper, more recently deposited layer, of course, would be less modified by profile development than the deeper parts. The character and age of the whole deposit, for that matter, would greatly influence the character of the soil. Most of the material in each profile appears to be derived from granite. Profiles D and E have distinct clay layers in the subsoil horizons. Although from the field evidence there is some question as to whether the clay was formed as the soil developed or whether it was water-deposited, there are indications that the clay has been developed in place as a result of soil-forming processes. Profile F has no clay layer and appears to be composed of more recently deposited stratified layers of coarse material.

In an attempt to discover the source of the formation of so large a proportion of clay in certain horizons, a preliminary mineralogical examination of the whole soil and of the sand fractions of a few profiles was made. This examination showed, as Brown and Drosdoff (*5*) have reported, that alteration of the feldspar is marked and the less easily decomposable minerals have also been altered. It was evident, however, that, although losses have occurred only minor differences in the mineralogy of the profiles are distinguishable.

While the mechanical analysis was being made, it was found that the coarse particles of the partly weathered biotite mica exfoliated readily when treated with hydrogen peroxide (*11*). This was caused by the presence, between the mica plates, of manganese dioxide, which decomposed the peroxide and caused exfoliation. This resulted in an increase in the coarse separates and a consequent decrease in the finer fractions, owing to the cohesion of the exfoliated plates of mica when the soil was heated after the peroxide treatment. However, the differences were not significant because of the relatively small percentage of this hydrated biotite mica.

The results of chemical analyses of the soils (table 2) indicate their granitic origin, although the soils of profiles D, E, and F are derived from transported alluvial fan material. The silica seldom exceeded 70 percent and was never less than 60. The sesquioxides and the bases fall well within the percentages occurring in granites. All parts of the profiles have been altered by weathering. Hydrolysis of the minerals has taken place, and the soluble material has been removed from the residue by the small amount of water that percolates through

the soil during the rainy season, as indicated by the small amount of soluble salts present. The soils are, nevertheless, alkaline throughout. The pH value was never less than 7.4 nor greater than 9, and was usually about 8. The lowest alkalinity usually occurred in the claypan horizons, where weathering tended to be greater than in other parts and the soluble bases had been removed.

The chemical analyses of the soils of the alluvial fans do not always show the effect of degradation of the primary minerals in the more continually moist claypan zone. The heterogeneous material of the alluvial fan is, however, largely derived from granitic sources, but the irregularities of deposition, in amount, time, and place, produce a condition that is only imperfectly overcome in the formation of a profile.

The results of chemical analyses of the colloids are presented in table 3. Decomposition of the primary minerals of the soil, pronounced losses of silica and sodium by leaching, and consequent increases of the sesquioxides, magnesium, and the ignition loss of the colloids, as compared with the analyses of the soils in table 2, are immediately apparent. Contrary to expectation, the low rainfall of the desert has supplied sufficient moisture for hydrolysis of the parent minerals and an effective seasonal leaching of the products in times of great supply. Marbut [3] has suggested that in regions of low rainfall soil characteristics may be produced similar to those of more humid areas if the rain is concentrated in certain seasons. Also Nikiforoff (*26*) has pointed out that the surface layer of the Desert soils dries quickly after rains and may form a mulch to keep the subsoil moisture from evaporating.

In these soils the field evidence is not altogether clear as to the relationship of the surface horizon to the rest of the profile, but it is certain that some movement of the surface material has taken place. Water has occasionally flowed over the soil, but most of the time the bare surface, protected only by scattered, scrubby vegetation, is exposed to the ravages of the desert winds. Apparently the composition of the colloids has not been appreciably altered by any transfer that has taken place. No sharp break in composition occurs in the profiles, and each horizon is apparently related to its neighbor. The colloids may, therefore, be examined as in normal soil profiles.

The colloid composition varies little with the intensity of claypan development in both the residual soil profiles derived from granite and the alluvial fan soils. As sodium is low, it is evident that the colloids are saturated with other bases, the carbonates have not been removed from the lower parts of the profiles, the soils are alkaline, and the silica lost has probably been removed as a sodium silicate.

The formula weight ratios (table 4) show a certain uniformity throughout this group of Mojave Desert profiles. The silica-sesquioxide ratios ranged from 2.46 to 3.27. The difference was less in any one profile. If this ratio is corrected for the free iron oxide present in profiles A and D, it is found to be slightly higher in each horizon, ranging from 2.6 to 3.4. It usually increased in the deeper, less weathered parts of both the residual and alluvial profiles. If, however,

[3] C. F. MARBUT. SOIL GENESIS AND CLASSIFICATION. U. S. Dept. Agr. Grad. School Lectures No. 10 6 pp.) and No. 30 (32 pp.). 1928. [Mimeographed.]

TABLE 2.—*Chemical analyses of Mojave Desert soils and parent rock materials* [1]

DERIVED FROM GRANITE

Profile and sample No.	Horizon	Depth	SiO_2	Fe_2O_3	Al_2O_3	MgO	CaO	K_2O	Na_2O	TiO_2	MnO	P_2O_5	SO_3	Ignition loss	Total	Organic matter	CO_2	N	Soluble salts	pH [2]
Profile A:		*Inches*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*P.p.m.*	
C3419	1	0-2	65.10	4.19	18.43	1.08	2.67	2.37	2.73	0.71	0.10	0.24	0.00	2.22	99.84	0.50	0.00	0.05	340	7.4
C3420	2	2-5	62.05	6.32	18.72	1.91	2.08	2.48	2.22	.70	.08	.15	.00	3.61	100.32	.25	.00	.05	140	7.4
C3421	3	5-11	62.05	6.42	18.72	1.57	2.30	2.38	2.10	.75	.13	.15	.00	3.65	100.22	.32	.00	.03	<140	7.6
C3422	4	11-14	61.10	6.98	18.33	1.51	2.95	2.28	1.99	.77	.11	.31	.00	3.88	100.21	.18	.27	.03	530	8.4
C3423	[3]	14-34	67.58	3.29	17.09	.85	2.95	3.17	2.40	.47	.08	.21	.00	1.47	99.56	.08	.00	.02	210	8.2
C3424	[3]	44-50	71.77	2.10	14.45	.69	3.90	2.24	2.42	.48	.05	.15	.00	1.35	99.60	-------	.47	-------	530	8.9
Profile B:																				
C3410	1	0-7	67.85	4.20	15.30	1.07	2.92	2.78	2.78	.61	.11	.10	.11	2.36	100.19	.08	.53	.02	320	8.4
C3411	2	7-10	66.70	4.82	15.53	1.59	3.16	2.72	2.40	.59	.09	.12	.00	2.71	100.43	.24	.59	.03	530	8.3
C3412	3	10-14	62.05	4.98	14.70	1.14	6.13	2.97	2.10	.53	.11	.15	.00	5.70	100.56	.31	3.11	.03	530	8.3
C3413	4	14-24	63.40	3.67	12.82	.75	7.77	2.40	2.28	.43	.07	.16	.04	6.32	100.11	.19	4.53	.03	210	8.6
Profile C:																				
C3401	1	0-6	65.36	4.03	17.80	1.18	2.40	3.33	2.26	.49	.06	.21	.01	2.51	99.64	.36	.00	.03	320	8.0
C3402	2	6-10	65.11	4.88	17.05	1.35	2.45	3.21	2.35	.66	.07	.17	.00	2.56	99.86	.29	.00	.02	320	8.0
C3403	3	10-17	61.40	5.44	16.68	1.45	4.16	2.92	2.28	.81	.08	.22	.00	4.21	99.65	.24	1.34	.02	320	7.9
C3404	4	17-26	61.59	5.88	17.33	1.50	3.88	2.84	2.72	1.05	.07	.31	.00	2.44	99.61	.16	.43	.02	420	8.1
C3405	[3]	36-48	60.08	4.00	15.18	1.06	7.35	2.52	2.82	.75	.07	.28	.00	5.50	99.61	.08	4.47	.01	210	8.2

DERIVED FROM ALLUVIAL FAN MATERIALS

Profile D:																				
C3395	1	0-3	69.54	3.85	14.20	0.94	2.13	3.29	2.76	0.50	0.06	0.13	0.02	2.49	99.91	0.66	0.00	0.04	460	8.1
C3396	2	3-12	70.23	3.76	14.45	.79	1.53	3.45	2.89	.46	.05	.12	.00	1.81	99.54	.24	.00	.03	140	7.8
C3397	3	12-24	66.67	5.12	15.85	1.07	1.66	3.20	2.28	.57	.06	.10	.00	2.98	99.56	.24	.00	.03	180	7.4
C3398	4	24-30	66.41	5.21	15.84	1.19	2.01	2.97	2.65	.53	.06	.08	.00	2.91	99.86	.17	.00	.03	210	7.8
C3399	5	30-36	68.06	4.71	15.42	1.13	2.17	2.88	2.49	.58	.06	.10	.00	2.43	100.03	.16	.00	.02	320	8.3
C3400	6	36-50	70.36	3.66	14.40	.94	2.07	3.21	2.76	.50	.05	.07	.00	1.79	99.81	.13	.00	.02	320	8.5
Profile E:																				
C3414	1	0-5	68.81	3.59	15.55	.76	1.74	3.23	3.06	.47	.19	.15	.00	2.38	99.93	.57	.00	.07	140	7.9
C3415	2	5-10	67.30	4.41	16.27	1.11	1.71	3.10	2.86	.57	.15	.11	.01	2.70	100.38	.26	.00	.03	140	7.7
C3416	3	10-20	67.35	3.82	17.28	1.09	1.52	2.57	2.06	.55	.07	.20	.00	3.15	99.65	.20	.00	.03	<140	7.6
C3417	4	20-34	65.70	4.21	17.00	1.20	2.57	2.44	2.33	.57	.06	.23	.00	3.37	99.68	.15	.44	.02	340	8.5
C3418	5	34-44	71.00	3.63	15.89	.93	1.84	2.47	2.51	.42	.06	.12	-------	1.56	100.43	-------	-------	-------	340	8.5
Profile F:																				
C3879	1	0-3	72.69	2.78	13.39	.72	2.15	2.79	2.37	.50	.04	.19	.00	1.80	99.42	.43	.00	.03	250	7.8
C3880-81	[4] 2-3	3-16	71.29	3.29	14.61	.90	2.30	2.66	2.02	.52	.05	.17	.00	1.88	99.69	.13	.00	.01	155	7.9
C3882-84	[4] 4-5-6	16-48	69.35	3.33	13.79	1.06	3.77	2.48	2.02	.50	.06	.15	.00	2.75	99.26	.12	.93	.01	320	8.5
C3885	7	48-60	61.47	4.87	15.65	2.15	5.40	2.21	2.21	.80	.06	.17	.00	4.87	99.86	.16	1.69	.01	550	8.7

[1] Organic matter by the combustion method ($CO_2 \times 0.471$), CO_2 of the carbonates, and N determined by J. H. Shimp.
[2] pH values determined by E. H. Bailey.
[3] Rotten rock.
[4] Composited.

TABLE 3.—*Chemical analyses of Mojave Desert soil colloids* [1]

DERIVED FROM GRANITE

Profile and sample No.	Horizon	Depth	Colloid extracted	SiO_2	Fe_2O_3	Al_2O_3	MgO	CaO	K_2O	Na_2O	TiO_2	MnO	P_2O_5	SO_3	Ignition loss	Total	Organic matter	CO_2	N
		Inches	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Profile A:																			
C3419	1	0–2	24	44.95	10.74	24.24	3.01	1.21	2.44	0.15	0.88	0.17	0.44	0.08	11.54	99.85	3.41	0.00	0.32
C3420	2	2–5	30	47.50	10.39	25.37	2.91	1.13	1.99	.03	.97	.19	.16	.00	9.47	100.11	.61	.00	.09
C3421	3	5–11	33	48.00	11.14	24.19	2.97	1.28	1.67	.06	.95	.11	.12	.00	9.49	99.98	.64	.00	.05
C3422	4	11–14	30	49.49	10.82	22.50	3.30	1.80	.87	.15	.87	.04	.20	.00	9.41	99.45	.50	.00	.06
C3423	5	14–34	6	50.12	9.70	22.04	3.41	2.51	.70	.13	.78	.08	.20	.00	9.96	99.63	.82	.27	.09
Profile B:																			
C3410	1	0–7	18	49.30	10.07	21.72	3.45	2.02	2.81	.21	.85	.12	.38	.05	9.40	100.38	1.58	.02	.17
C3411	2	7–10	27	51.45	9.40	21.70	3.84	1.90	2.06	.27	.70	.06	.14	.02	8.71	100.25	.79	.00	.10
C3412	3	10–14	26	48.35	8.68	20.42	3.70	5.49	1.75	.21	.61	.05	.09	.02	11.30	100.67	.29	3.56	.10
C3413	4	14–24	28	46.30	8.76	19.02	3.39	6.48	1.42	.19	.72	.05	.38	.01	13.23	99.95	.36	4.73	.11
Profile C:																			.11
C3401	1	0–6	18	50.61	9.15	22.36	3.35	2.45	1.29	.22	.72	.08	.37	.02	9.20	99.82	1.17	.00	
C3402	2	6–10	12	51.30	9.24	21.23	3.50	2.43	1.36	.09	.73	.04	.22	.00	9.35	99.49	.96	.00	.09
C3403	3	10–17	15	47.44	7.86	20.83	3.28	6.35	1.18	.04	.68	.04	.28	.00	11.88	99.86	.86	3.09	.08
C3404	4	17–26	11	49.09	9.15	21.40	3.03	4.16	1.06	.09	.68	.04	.35	.00	10.82	99.87	1.28	1.18	.11

DERIVED FROM ALLUVIAL FAN MATERIALS

Profile D:																			
C3395	1	0–3	21	51.07	8.96	20.90	3.44	1.83	1.98	0.43	0.52	0.16	0.12	0.02	10.42	99.85	2.81	0.00	0.28
C3396	2	3–12	32	49.28	9.25	24.36	2.68	1.31	2.74	.17	.51	.08	.14	.00	8.96	99.48	1.20	.00	.13
C3397	3	12–24	29	49.17	9.80	24.10	2.65	1.45	1.36	.11	.58	.06	.12	.00	10.02	99.42	.82	.00	.10
C3398	4	24–30	29	49.50	10.06	24.08	2.73	1.77	1.09	.26	.59	.06	.06	.00	9.84	100.04	.92	.00	.12
C3399	5	30–36	23	50.55	10.12	23.39	2.79	1.93	1.16	.18	.57	.08	.06	.00	9.39	100.22	.82	.00	.11
C3400	6	36–50	22	51.37	10.18	21.81	3.20	2.01	1.44	.18	.50	.08	.14	.00	8.71	99.62	.96	.00	.10
Profile E:																			
C3414	1	0–5	26	49.30	10.63	23.17	3.06	1.53	2.62	.29	.69	.15	.29	.03	9.13	100.89	1.35	.00	.14
C3415	2	5–10	32	50.20	10.41	23.47	2.91	1.41	2.52	.10	.68	.08	.16	.08	8.50	100.52	.82	.00	.09
C3416	3	10–20	61	50.42	9.69	23.33	2.95	1.32	1.77	.02	.70	.09	.08	.01	9.14	99.52	.52	.00	.06
C3417	4	20–34	40	50.45	10.06	22.50	3.08	1.81	1.63	.06	.87	.07	.12	.00	9.51	100.16	.64	.00	.08
C3418	5	34–44	.....	50.80	10.22	22.20	3.22	1.92	1.62	.11	.67	.09	.21	.00	9.07	100.13	.68	.00	.09
Profile F:																			
C3879	1	0–3	.....	46.27	9.40	23.14	3.46	1.83	2.33	.26	.44	.08	.24	.00	13.12	100.57	4.20	.08	.38
C3880–81	[2] 2–3	3–16	.....	48.94	8.96	23.86	3.47	1.84	2.51	.25	.44	.05	.18	.00	9.48	99.98	1.24	.02	.13
C3882–84	[2] 4–5–6	16–48	.....	49.11	7.79	22.03	3.88	3.86	2.48	.25	.38	.06	.18	.00	10.83	100.85	.89	1.65	.14
C3885	7	48–60	.....	48.19	8.01	20.19	4.46	5.15	1.72	.28	.36	.06	.22	.00	11.15	99.79	1.00	2.51	.11

[1] Organic matter determined by the combustion method ($CO_2 \times 0.471$). CO_2 of the carbonates and nitrogen determined by J. H. Shimp.
[2] Composited.

iron oxide, whether free or combined, is wholly ignored and silica is compared directly with alumina, it is noted that about the same uniformity prevails throughout the samples, the ratios ranging from 3.16 to 4.15. With few exceptions a fairly regular increase with depth occurred irrespective of the amount of clay in the profile. Although the change was not great, it indicated that the small amount of water that penetrated the soils had removed silica from the upper parts of most of the profiles.

TABLE 4.—*Derived data: Mojave Desert colloids*

Profile and sample No.	Horizon	Depth	Formula ratio						Combined water [4]	Combined water of the soil acid [5]
			SiO_2 / $Fe_2O_3.Al_2O_3$	SiO_2 / Fe_2O_3[1]. Al_2O_3	SiO_2 / Al_2O_3	SiO_2 / Total bases[2]	SiO_2 / H_2O[3]	H_2O[3] / Al_2O_3		
DERIVED FROM GRANITE										
Profile A:		*Inches*							*Percent*	*Percent*
C3419	1	0–2	2.46	2.6	3.16	6.01	1.30	2.43	8.13	10.64
C3420	2	2–5	2.52	2.7	3.18	6.94	1.30	2.71	8.86	10.98
C3421	3	5–11	2.61	2.8	3.52	6.94	1.32	2.83	8.85	11.00
C3422	4	11–14	2.86	3.0	3.74	6.56	1.33	2.82	8.91	11.24
C3423	5	14–34	3.02	3.2	3.86	6.65	1.35	2.86	8.77	11.31
Profile B:										
C3410	1	0–7	2.98	3.1	3.85	5.30	1.34	2.87	8.20	11.17
C3411	2	7–10	3.16	3.3	4.03	5.52	1.44	2.80	7.92	10.80
C3412	3	10–14	3.15	3.3	4.01	4.96	1.39	2.88	7.45	11.16
C3413	4	14–24	3.19	3.4	4.12	5.07	1.40	2.94	7.14	11.52
Profile C:										
C3401	1	0–6	3.05	3.2	3.85	5.86	1.41	2.73	8.17	10.88
C3402	2	6–10	3.21	3.4	4.10	5.82	1.39	2.95	8.39	11.14
C3403	3	10–17	3.12	3.3	3.87	5.72	1.34	2.89	8.13	11.52
C3404	4	17–26	3.06	3.2	3.85	6.03	1.36	2.83	8.36	11.26
DERIVED FROM ALLUVIAL FAN MATERIALS										
Profile D:										
C3395	1	0–3	3.26	3.4	4.15	5.83	1.39	2.99	7.61	10.57
C3396	2	3–12	2.76	2.9	3.42	6.72	1.45	2.36	7.76	10.08
C3397	3	12–24	2.75	2.9	3.46	7.60	1.32	2.62	9.20	11.24
C3398	4	24–30	2.75	2.9	3.49	7.14	1.35	2.59	8.92	11.11
C3399	5	30–36	2.88	3.0	3.67	6.91	1.21	3.03	8.57	12.67
C3400	6	36–50	3.07	3.2	3.99	6.64	1.28	3.12	7.79	12.07
Profile E:										
C3414	1	0–5	2.78	2.9	3.60	6.04	1.43	2.52	7.78	10.41
C3415	2	5–10	2.72	2.9	3.45	6.66	1.52	2.27	7.62	9.97
C3416	3	10–20	2.90	3.1	3.67	7.26	1.41	2.60	8.62	10.76
C3417	4	20–34	2.96	3.1	3.81	6.62	1.24	3.07	8.87	12.24
C3418	5	34–44	3.00	3.2	3.87	6.36	1.41	2.75	8.39	10.85
Profile F:										
C3879	1	0–3	2.70	2.9	3.39	5.20	1.21	2.80	8.84	11.95
C3880–81	[6] 2–3	3–16	2.77	2.9	3.43	5.48	1.34	2.56	8.22	11.05
C3882–84	[6] 4–6	16–48	3.08	3.2	3.78	5.18	1.32	2.86	8.29	11.66
C3885	[6] 7	48–60	3.27	3.4	4.10	4.86	1.37	2.99	7.64	11.44

[1] Combined iron oxide.
[2] Oxides of Mg, Ca, K, and Na.
[3] Ignition loss less the organic matter, plus the water equivalent of the bases.
[4] Ignition loss less the organic matter.
[5] Ignition loss less the organic matter plus the water equivalent of the bases, corrected for the organic matter and carbonate content.
[6] Composited.

The silica-base ratio was low and showed only slight variation in each profile as well as in the whole group of samples, irrespective of the origin of the materials on which the soils had formed. Although removal of bases, especially sodium, had occurred during the hydrolysis and degradation of the primary minerals, a large part of the magnesium and most of the other bases still remained in the colloid.

The combined water in the whole group of colloids ranged from 7.14 to 9.20 percent. The average was 8.30. The variation from the mean was 18.9 percent. In profiles B and F the combined water was higher in the surface horizon than in other parts. In the other profiles the combined water tended to be greater in the deeper, cooler parts. In none of the profiles did it follow closely the clay content of the soils.

The combined water of the soil acid postulated by Byers (*7*) showed less variation than the combined water alone. The variation from the mean was 13.4 percent, and the tendency to increase with depth occurred more regularly and more often than in the combined water. The highest values always occurred in the deeper parts of those profiles containing a claypan. The hypothetical combined water of the soil acid is obtained by adding the water equivalent of the bases to the combined water and correcting the sum for organic matter. It carries no implication as to how the various constituents are combined in the component clay minerals of the soil.

A comparison of either silica or alumina with the combined water of the soil acid shows still greater uniformity than the other ratios throughout all of the profiles. The average of the ratio of the combined water of the soil acid to silica was 1.35 and to alumina, 2.77. The variation from the mean of the first was 11.5 percent and of the second, 12.6. The variation within any one profile was very much less. These ratios indicate a remarkable uniformity in the relationships between the three principal constituents of the colloids irrespective of the amounts of each in the component minerals in the colloid.

The calculation of the combined water of the soil acid rests on the assumption that all of the bases present are replaceable by hydrogen on complete hydrolysis of the colloid complex. Recent evidence based on X-ray examinations indicates that a part of some of the bases, particularly magnesium, exists as a substituent for aluminum in the lattice structure of the clay minerals (*14*, *24*, *25*, *28*). Consequently the assumption that hydrogen can replace all of the bases is probably not valid. Moreover, the base-exchange relations of the colloids (table 5) show that only about 11 percent of the total magnesium is exchangeable. The work of Kelley, Dore, and Brown (*19*) indicates that, upon grinding, all of the bases of the colloids can be extracted with ammonium acetate. This does not necessarily show that an increase in the base-exchange capacity occurs and that these bases are actually replaceable by hydrogen. Probably this increase of bases, removed after grinding, is accompanied by the disintegration of a part of the colloid complex, as is indicated by Drosdoff (*10*).

These various relationships indicate that the principal constituents of the colloids are essentially the same in all the profiles presented. It is also evident that profile variations of the colloid composition are slight irrespective of the remarkable quantity of clay in parts of most of the profiles. It appears certain, however, that the composition of the colloid is only slightly influenced by many factors that cause profound differences in the distribution of the clay and other morphological properties of the soils. The changes in morphology are greater than expected in soils receiving so little rainfall.

Another point of interest is the fact that the colloid extracted from the rotten granite rock of profile A, horizon 5, has essentially the same chemical and mineralogical composition as the claypan horizon containing over 35 percent of clay. It was surprising to find that the apparently slightly weathered granite contained as much as 14 percent of clay. As the material had been broken and powdered in the course of collecting the sample, a further mechanical analysis was not made, but from observation it appeared that the material was largely coarse sand and gravel with the clay particles sticking to the coarser particles. Although rock decomposition in this profile is far from complete, apparently the colloids formed have reached an equilibrium with their environment.

Information about the base status of representative colloids was obtained by determining the exchangeable-base and base-exchange capacity of one of the residual soils (profile A) and one of the alluvial fan soils (profile D). These results are shown in table 5. They indicate that the colloids are practically all saturated with bases and that those which are replaceable consist almost entirely of calcium and magnesium. The exchangeable sodium and potassium were very low. In profile A the ratio of exchangeable calcium to exchangeable magnesium was approximately 2 to 1 whereas in profile D it was roughly 4 to 1.

The calcium present in the colloids was over 83 percent exchangeable in all except the lowest layer in each profile. It appears that the horizons having the most clay have less calcium in the nonexchangeable form. This indicates that hydrolysis has proceeded further in the claypan than in other layers. Only about 10 to 15 percent of the total magnesium, and a very small fraction of the potassium, are apparently present in the exchangeable form. These bases, therefore, must be firmly bound and must be present largely as a more permanent part of the clay minerals.

TABLE 5.—*Replaceable bases in colloids*

PROFILE A

Laboratory No.	Horizon	Ca		Mg		Na		K		Total replaceable bases	Base-exchange capacity
		Milliequivalents per 100 gm.	Percent of total CaO	Milliequivalents per 100 gm.	Percent of total MgO	Milliequivalents per 100 gm.	Percent of total Na_2O	Milliequivalents per 100 gm.	Percent of total K_2O	Milliequivalents per 100 gm.	Milliequivalents per 100 gm.
C3419	1	37.2	86	22.5	15	1.2	20	0.2	1	61.1	.....
C3420	2	36.5	90	15.6	11	.9	100	.3	1.5	53.3	.....
C3421	3	43.9	96	24.4	16	1.0	50	.3	2	69.6	.....
C3422	4	56.2	87	22.1	13	.9	20	.2	2	79.4	.....
C3423	5	47.0	53	19.3	11	.....	.....	.....	.....	66.3	.....

PROFILE D

C3395	1	55.2	84	11.6	7	1.0	8	0.3	1.5	68.1	74.3
C3396	2	40.3	87	12.2	9	.....	.....	.....	.....	52.5	57.7
C3397	3	50.4	97	13.8	10	.8	20	.2	1.5	65.2	67.3
C3398	4	62.4	99	13.6	10	.7	10	.3	3	77.0	75.3
C3399	5	60.5	87	13.9	10	.9	16	.9	6	76.2	77.4
C3400	6	56.3	79	14.1	9	.8	11	.8	6	72.0	78.3

In order to obtain more definite information on the clay mineral content of the colloids, X-ray analyses were made of those from profiles A and D.[4] The clay minerals present were found to be essentially the same in all of the samples and consisted of about 25 percent of kaolinite (halloysite) and 70 to 80 percent of what can best be described as a mixed-layer mineral. This type of clay mineral has been discussed by Hendricks and Alexander (*15*). It was shown by Alexander, Hendricks, and Nelson (*1*) to be a composite mineral of montmorillonite and hydrous mica. That such minerals can be formed by interstratification of different kinds of layers has been shown by Gruner (*13*). Though this type of mixed mineral is difficult to recognize, it is probably a common constituent of soils (*15*).

The montmorillonite layers are essentially the same in composition as the hypothetical "pyrophillic acid" postulated by Byers (*7*). The ideal formula has a silica-alumina ratio of 4 to 1 and is modified by isomorphous replacements, as discussed later.

The hydrous mica type of clay mineral has recently been recognized as a common constituent of soil colloids by a number of investigators (*11, 12, 23*). The ideal formula may be considered to be the same as that given for mica but with less potassium and more water. The nonexchangeable potassium is firmly bound in the crystal lattice of the hydrous mica. It has been pointed out that the percentage may vary considerably without changing the structure of the mineral (*15*). In the colloids examined here, the potassium oxide content ranged from 0.70 to 2.74 percent, whereas the amount of the mixed-layer mineral, hydrous mica-montmorillonite, remained approximately the same.

The nonexchangeable magnesium of the colloid may be present in either the hydrous mica or the montmorillonite layers. The variation in the amount depends on how much isomorphous replacement of magnesium for alumina occurs. That magnesium can replace aluminum in the lattice structure in the ratio of 3 of magnesium to 2 of aluminum is now generally accepted (*14, 24, 25, 28*). The combined iron may also substitute for aluminum in the internal structure of the clay mineral.

As stated above, about 25 percent of the colloids of these soils is composed of kaolinite or halloysite, which is similar in composition to the hypothetical "halloysitic acid" postulated by Byers (*7*). The ideal composition has a silica-alumina ratio of 2 to 1. There is very little isomorphous replacement in this type of clay mineral, since it is largely restricted to mutual substitutions of aluminum and silica. Apparently the X-ray data indicate that kaolinite and halloysite are very similar and belong to the family of kaolin minerals (*30*).

The reddish-brown color of the colloids indicates the presence of some free iron oxide. The amount was determined by the hydrogen sulphide method described by Truog et al. (*32*) on the colloids of soils A and D. The results are presented in table 6. It was found that about one-fourth of the iron near the surface, and about one-fifth in deeper parts of the profile, is free oxide; the rest is combined as a silicate. As colloids from profiles A and D are similar to the others in composition, approximately the same amount of free iron oxide is

[4] These analyses were made by S. B. Hendricks, of the Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture.

assumed to be present in the colloids of comparable parts of the other profiles. Reddish-brown soils of the humid regions have a much larger proportion of free iron oxide. Therefore it appears that under conditions of low rainfall the weathering processes result in the segregation of a comparatively small amount of free iron oxide, most of the iron remaining in the combined form. The fact that approximately one-fourth of the total iron is present as free oxide suggests that its formation is coincident with the formation of kaolinite, as this clay mineral comprises about one-fourth of the colloid. The kaolin minerals do not contain appreciable amounts of iron or magnesium.

TABLE 6.—*Percentage of iron oxide in the colloids*

Laboratory No.	Horizon	Depth	Total	Free	Proportion free
		Inches	*Percent*	*Percent*	*Percent*
PROFILE A, DERIVED FROM GRANITE					
C3420	2	2–5	10.39	2.8	27
C3421	3	5–11	11.14	2.2	20
C3422	4	11–14	10.82	2.4	22
C3423	5	14–34	9.70	2.0	21
PROFILE D, DERIVED FROM ALLUVIAL FAN MATERIALS					
C3396	2	3–12	9.25	2.6	28
C3397	3	12–24	9.80	2.0	20
C3398	4	24–30	10.06	2.1	21
C3400	6	36–50	10.18	1.9	19

Aside from the predominant mixed-layer mineral, hydrous mica-montmorillonite, the kaolinite, and the small amount of free iron oxide as determined by chemical analysis, there is also the doubtful possibility of a small percentage of quartz present. Quartz and free iron oxide are present in amounts too small to be detected with certainty by X-ray analysis.

It is interesting to note that the coarse biotite-vermiculite mica particles of the partially weathered granite of the residual soils gave essentially the same X-ray pattern as the predominant clay mineral of the colloid. This suggests that the micas are more important in the formation of clay from granite than has been previously supposed. It has been generally assumed that because the feldspars are by far the most predominant mineral group in granite, the clay minerals are formed largely from them, either directly or indirectly. The coarse mica in the rock contains 5.5 percent of potassium oxide and 7.1 percent of combined water. With further hydrolysis, potash would be replaced by water and thereby approach the composition of the soil colloid. Denison, Fry, and Gile (*9*) determined the changes in composition of the coarse mica particles undergoing weathering and with mineralogical control showed a continuous variation from the pure micas to the highly weathered micas, which are similar to the hydrous mica of the soil colloid.

The laboratory data presented corroborate the suggestion of Nikiforoff (*26*) that the claypan in the Mojave Desert soils is formed in place by hydrolysis of the primary minerals of the soil. Evidence of this is found in the remarkable uniformity of the chemical and mineral-

ogical composition and the amount of degradation of the colloids derived from both residual granite and alluvial fan materials in the region of extremely low rainfall. The coarse mica is of the same composition as the predominant clay mineral colloids of the soil. The alluvial fan profiles appear to be as well developed as the residual granite and do not reflect the conditions of deposition. Profile development occurs without significant change in the colloids. This indicates that the claypan is developed in place and is genetically related to the other horizons. The amount of clay in any horizon is dependent on the moisture in the soil, the age of the soil, and the freedom of the soil from erosion.

SUMMARY

Six soil profiles from the Mojave Desert have been studied. Of these, three are residual soils derived from granite; three are derived from alluvial fan material; five have well-developed claypan horizons, and one has none. They are from a comparatively restricted area near Mojave, Calif., and occur at 2,700 to 3,000 feet above sea level. The mean annual rainfall presumably is approximately 5 inches and the mean annual temperature is about 65° F.

The results show that the chemical alteration of claypan soils is greater than the moisture supplied by the rainfall of the desert is expected to produce. Neither the chemical nor the mineralogical composition of the colloids is influenced by the formation of a claypan in these desert soils. The colloids are saturated with bases, chiefly calcium and magnesium. Of the total iron, 25 percent is present as free iron oxide.

The X-ray data indicate that about 75 percent of the colloids is composed of a mixed-layer mineral of hydrous mica and montmorillonite similar to the coarse weathered mica in the soil. About 25 percent is kaolinite (halloysite).

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EFFECT OF FEEDING REPEATED SMALL DOSES OF SELENIUM AS SODIUM SELENITE TO EQUINES [1]

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INTRODUCTION

Much loss has been occasioned to the livestock industry through chronic selenium poisoning in a number of sections of the north-central Great Plains. Animals fed grain and roughage grown in these areas manifest, after varying lengths of time, a more or less characteristic syndrome known as alkali disease. The symptoms most frequently described are unthriftiness, loss of weight and hair, and abnormal growth of the horn of the hoof with attendant lameness. In poultry, failure to reproduce because of poor hatchability of eggs or the occurrence of weak, deformed chicks, has also been observed. Considerable uncertainty existed as to the cause of this condition until about 7 years ago, when selenium was definitely associated with the disease.

Since that time many experiments have been carried out to test the action of selenium on various species of animals. Both seleniferous feed and different organic and inorganic compounds of selenium have been used for this purpose. As a consequence, much information has been obtained on the toxicity and pathological effect of selenium on laboratory animals, such as rats, rabbits, cats, and dogs, and in a few instances on the larger domestic animals. Two experiments have been reported in which swine were the test animals. In one of these (*6*),[2] seleniferous corn grown in affected areas was fed; in the other (*3*), sodium selenite was added to the feed to give different concentrations of selenium. Typical symptoms of selenium poisoning were produced in some of the animals in each of these experiments. In the light of the results of this work and since there is no known information on the experimental feeding of repeated small doses of selenium to equines, it was decided to investigate this phase of the problem. The work was done during 1937 and 1938 at the Animal Disease Station, Beltsville, Md.

EXPERIMENTAL PROCEDURE

Because of the limited number of animals available for the experiment, approximately the same concentration of selenium was given to each animal. In the beginning of the experiment, two horses and a mule were used, and some time later a third horse was added. Of the three horses, one (No. 962) was a mare about 12 years old, the second (No. 980) was a gelding about 5 to 6 years old, and the third (No. 1023) was an aged gelding which had been used previously in an equine encephalomyelitis experiment. The mule, a gelding (No. 1034), was also an aged animal which had passed through other disease experiments. However, all the animals were apparently in good health when this experiment began.

[1] Received for publication December 29, 1939.

[2] Italic numbers in parentheses refer to Literature Cited, p. 368.

About 5 weeks before the selenium was added to the feed, horses 962 and 980 and the mule were placed in a small stable with four stalls and an exercise yard about 100 feet square. There were a few weeds and several shade trees in the yard but no vegetation for the animals to eat. The animals were watered only when they were turned out for exercise, which was from 9 a. m. to 4 p. m. daily. The water was obtained from the regular supply at the Animal Disease Station at Beltsville, Md.

The feed, consisting of No. 2 oats and No. 2 timothy hay, was obtained from the station supply, which was used in feeding all the horses kept on the premises, both for work and experimental purposes. After some experimentation, it was found that 5 pounds of oats with 20 pounds of hay for the horses and 5 pounds of oats with 15 pounds of hay for the mule were adequate rations which would be cleaned up regularly. These rations were continued for the duration of the experiment. When the third horse was added, it received 5 pounds of oats and 15 pounds of hay. Any oats and hay left in the mangers following the addition of sodium selenite to the feed were weighed after each feeding.

At the beginning of the experiment, selenium in the form of sodium selenite was given at the rate of 24 parts per million of feed to the horses and 17.3 parts per million to the mule. This proportion was obtained by mixing 35 cc. of an M/10 solution of sodium selenite (17.3 gm. per liter) in distilled water with the 5 pounds of oats for the horses; 21 cc. of the solution was given to the mule in the same manner. The animals ate the selenized oats readily at first but after about 2 months refused them and ate only hay. The method of administration was then changed to drenching each animal with the sodium selenite solution by means of a dose syringe after the oats had been eaten in the morning. Hay was fed at night while the animals were in the stable. To facilitate drenching, an M/5 solution of sodium selenite (34.6 gm. per liter) was made up and the volume of the dose reduced by half, namely, 17.5 cc. to the horses and 10.5 cc. to the mule. This method was continued for about 4 months, making a total of 6 months that the horses ingested selenium equivalent to 24 parts per million of feed and the mule, 17.3 parts. Then the dose was doubled with the result that each horse received 48 parts, and the mule 34.6 parts, of selenium per million parts of feed, 35 cc. of an M/5 solution of sodium selenite being given to the horses and 21 cc. to the mule.

About 4 months later another change was made in the method of administering the selenium to the horses, the mule having died in the meantime. This was necessitated by the fact that both of the horses gradually refused to swallow the solution and part of it was lost at each drenching. This difficulty was overcome by diluting the daily dose of M/5 solution of sodium selenite to 700 cc. with water. Of this diluted solution, 150 cc. was sprinkled on the oats and the remainder on the hay. In order to obtain an even distribution of the solution in the hay, as well as to facilitate weighing, the hay was chopped into lengths of about 1½ inches. The horses ate the chopped hay as well as when it was in its original form, and they continued to eat both the selenized oats and hay satisfactorily until shortly before death. Since the condition of both horses seemed to have remained relatively static even after they had ingested 48 parts of selenium per million of feed for

about 9 months, the dose was increased to about 96 p. p. m., 70 cc. of an M/5 solution of sodium selenite being diluted to 700 cc. with water and then mixed with the feed as previously described. This quantity was given until the experiment was terminated by the death of the animals.

About a month after the last increase in the dose of selenium, horse 1023 was placed on experiment. It was given the same dose of sodium selenite solution as the other two horses, although it was eating 5 pounds less hay. The selenium level for this animal was, therefore, approximately 115 parts per million of feed. Three weeks later the concentration was reduced to 96 p. p. m. by giving 56 cc. of the M/5 solution of sodium selenite diluted to 700 cc. with water. This dose was continued for 2 weeks, or until the horse died.

In order to determine what concentration of selenium might be produced in the blood by feeding repeated small doses of selenium, samples of blood were taken from each animal before the experiment began and each week thereafter. The blood was taken from the jugular vein and at approximately the same time of day on each occasion. Fecal and urine samples were collected at irregular intervals and at the same time as the blood samples. The feces were removed directly from the rectum. Urine was obtained from the mare (No. 962) by means of a catheter. The first few urine samples were drawn from the geldings (Nos. 980 and 1034) by passing the catheter, but later it was found that the animals could be induced to urinate by brisk massage of the bladder and the urine was caught in a clean pan. In addition to the analysis of these specimens, selenium determinations were made on the body tissues and fluids which were collected at the time of autopsy.

EXPERIMENTAL RESULTS

HORSE 962

Horse 962 received a small dose of selenium daily for a period of 17 months and 12 days before death occurred. At the beginning of the selenium-feeding period (July 26, 1937) the animal weighed 1,000 pounds, whereas at the time of death its weight had decreased to 660 pounds. The weekly changes in the weight of the animal are shown in figure 1. Although there was considerable fluctuation in the weight from week to week, a definite downward trend did not appear until about 3½ months after the experiment began. From that time on there was a gradual decrease until 3 weeks before death, when the weight decreased abruptly. The animal's appetite remained good until the last 10 days of the experiment, when practically all the oats and hay were left untouched.

Although the level of selenium given in the feed to this animal was relatively high and had been found to be effective in producing typical symptoms of chronic selenium poisoning in swine (*3*), pronounced physical changes such as have been described in horses grazing on seleniferous soil (*2*) were not observed. The symptoms, other than emaciation and listlessness, were rather mild. Figure 2, *A*, shows horse 962 as it appeared 1 week after the beginning of the experiment, and *B*, the same animal 3 weeks prior to death, or about 17 months later. Up to that time the appetite had been good, but there had been a loss of about 150 pounds in weight, the coat was staring, and

when the animal was aroused it moved slowly with an unsteady gait. The hair was loose in the mane and tail, particularly in the mane, which contained much matted hair. There was no evidence of loosening of the hair until about 6 months after the beginning of the experiment. From that time on, the hair could be pulled out readily but there was never any tendency for it to shed abnormally.

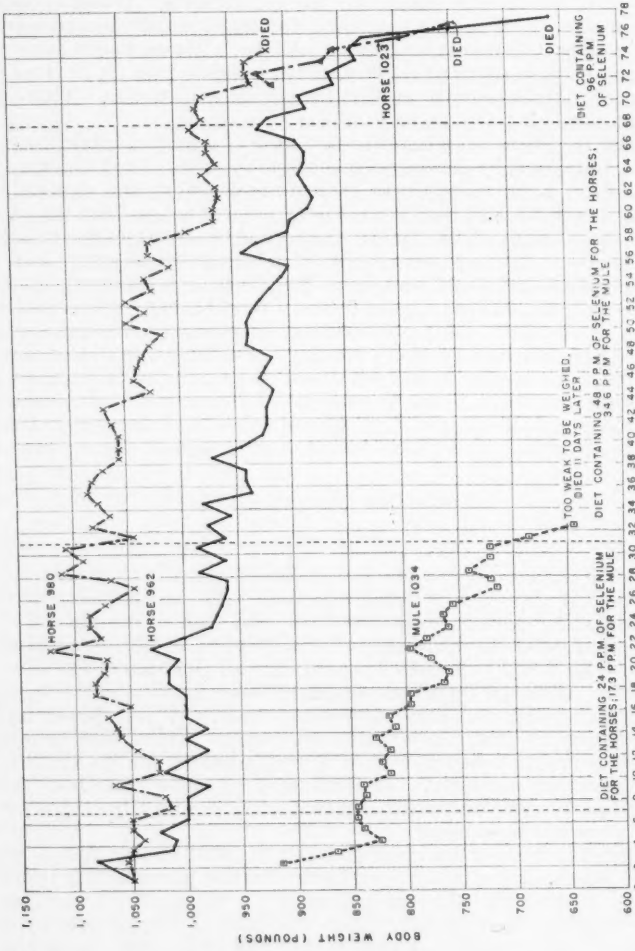
HORSE 980
HORSE 962
MULE 1034
HORSE 1023
DIED
DIED
DIED
TOO WEAK TO BE WEIGHED. DIED 11 DAYS LATER
DIET CONTAINING 48 PPM OF SELENIUM FOR THE HORSES; 346 PPM FOR THE MULE
DIET CONTAINING 24 PPM OF SELENIUM FOR THE HORSES; 173 PPM FOR THE MULE
DIET CONTAINING 96 PPM OF SELENIUM
BODY WEIGHT (POUNDS)
WEEKS

FIGURE 1.—Weekly changes in body weight of the experimental animals as a result of ingesting small repeated doses of selenium in the form of sodium selenite.

Changes in the horn of the hoofs were observed, but they were not outstanding. A year after the experiment began, there was considerable scaling of the horny wall of the hoofs for a distance of several inches below the coronary band. When the loose scales were removed, the horn had a white flaky appearance. In addition, the

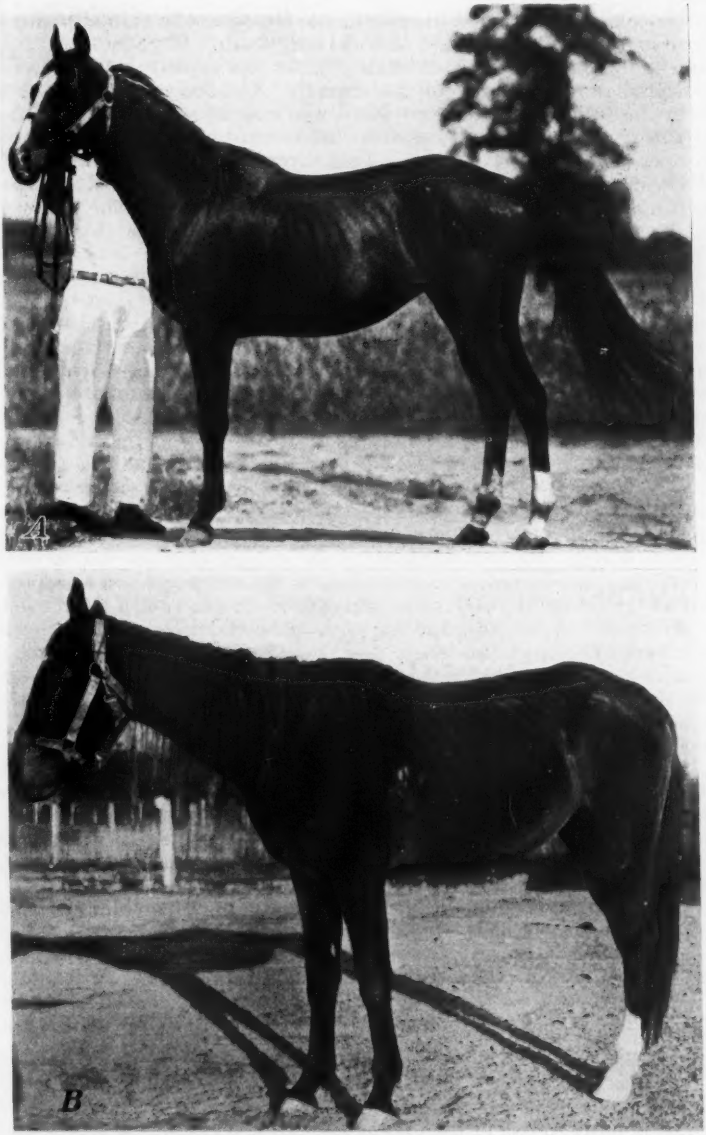

FIGURE 2.—*A*, Horse 962 as it appeared 1 week after the beginning of the selenium-feeding experiment, and *B*, after 17 months of selenium feeding. Note emaciation, drowsiness, and matted hair of mane in *B*.

horn seemed softer, as indicated by excessive wear of the hoofs. The latter condition occurred in spite of the fact that the animal was confined in a relatively small lot on soft sandy loam. Slight scaling of the wall first appeared about 3 months after the experiment began and persisted during the life of the animal. No separation of the hoof from the skin at the coronary band was ever observed. In addition to this change, the feet seemed to become sore at irregular intervals for periods of several weeks. This soreness was evidenced by the short stilted steps taken by the animal in walking and a constant shifting of the weight when standing. At such times the coronary band seemed to be slightly swollen and somewhat painful on palpation.

Another symptom which was manifested occasionally was extreme nervousness or sensitivity when blood samples were taken. As the needle was introduced into the jugular vein, the neck muscles would contract and almost shut off the flow of blood, whereas at other times the animal remained quiet and offered no resistance while being bled. One other peculiarity was observed during the course of the experiment. About 3 months after selenium feeding began, this horse, as well as horse 980 and mule 1034, was observed chewing on the wooden parts of the fence of the enclosure. Somewhat later the animals were seen licking the dirt in the yard, in spite of the fact that stock salt was accessible at all times. Other animals maintained on the same feed without selenium showed no evidence of depraved appetite.

Post-mortem examination showed the carcass of horse 962 to be in a very emaciated condition. The hair of the mane and tail was loose, and all four hoofs showed some flaking of the horn. Both the mucous membranes and the conjunctiva were pale and icteric. There was some emphysema of the lungs, due possibly to the labored terminal breathing. The fat of the heart was of a gelatinous consistency, the heart muscle was pale, and the endocardium was yellow and spotted with a few subendocardial hemorrhages. The liver was darker in color and somewhat smaller than normal. The surface of this organ showed many fibrinous tags and a prominent demarcation of the lobules, indicative of marked cirrhosis. On sectioning, the cut surface was yellowish chocolate in color and there was a marked increase in the connective tissue. A few subcapsular petechiae were present on the surface of the spleen, which was somewhat smaller than usual. When sectioned, the surface was dry, the pulp was dark and doughy, and there was an increase in the connective tissue. The kidneys were chocolate in color and the capsules were difficult to remove. A few hemorrhages were found in the cortex of the organ. The peritoneum was icteric and there was a slight enteritis.

HORSE 980

Horse 980 received the same quantity of feed and the same dose of selenium as horse 962. Although it weighed about 50 pounds more at the beginning of selenium feeding and remained slightly heavier throughout the course of the experiment, it died about 2 weeks before the latter animal. The changes in weight during the feeding period appear in figure 1. The weekly fluctuations in the weight of horse 980, as well as the general downward trend, although beginning a little later, fairly closely parallel those of horse 962. In horse 980,

however, the final precipitous decrease in weight was interrupted by the death of the animal. As in the first case, the appetite of horse 980 remained good until about 10 days prior to death.

The clinical picture was largely a replica of that of horse 962. In figure 3, *A*, horse 980 is shown as it appeared 1 week after the beginning of the experiment, and *B*, the same horse 3 days before death. By that time there had been a loss of about 200 pounds in weight and the animal was barely able to walk.

Changes in the character of the horn of the four hoofs just below the coronary band appeared about 2 months after the beginning of the experiment. A view of the front feet after the animal had been fed selenium for 1 year is shown in figure 4. There was distinct swelling of the coronary band (*a*), a roughened scaly formation on the horny wall about 1½ inches wide immediately below (*b*), and extreme wear and breaking away of the horn at the toes. A small portion of the horny wall that was present before the beginning of selenium feeding and that was distinguished from the selenized horn by a slight depression remained on the toes of both front feet. This ringed appearance was first noticed at the top of the hoofs shortly after the horn became roughened. Since the horse showed little disposition to move around a great deal from this time on, no further breakage occurred and the character of the horn showed little subsequent change. Periodic tenderness of the feet at irregular intervals was noticed in this animal also, although not always at the same time that it appeared in horse 962.

The hair in the mane and tail became loose at about the same time as in horse 962, and just before death the hair in both mane and tail was very loose. In addition, occasional periods of nervousness appeared as previously described and the same depravity of appetite was noted.

Post-mortem examination showed the carcass to be very thin. The blood was dark red in color. Aside from a slight emphysema and a few fibrinous tags on the visceral pleura, the lungs appeared more or less normal. The fat in the mediastinum was gelatinous. A few petechiae were present in the gelatinous fat along the coronary groove. Small subepicardial hemorrhages occurred in the region of the coronary vessels and larger ones on the right auricle. A few large hemorrhages were found on the right auriculoventricular valves. The heart muscle was pale and had a cooked appearance. The surface of the liver, which presented a few fibrinous tags, was yellowish in color and the lobules were hemorrhagic. On sectioning, the liver tissue was found to be of an ocher color and the lobules stood out distinctly. Numerous small hemorrhages occurred under the capsule of the spleen. There were also many ecchymotic hemorrhages in the omentum and mesentery. The cortex of the kidney contained some petechiae, the medulla was reddened, and the pelvis was filled with clear, gelatinous urine. The intestines showed some enteritis, and the peritoneum was reddened.

MULE 1034

Although mule 1034 received a slightly smaller quantity of selenium per day than did horses 962 and 980, it died much sooner, about 6½ months after the experiment began. The concentration of selenium

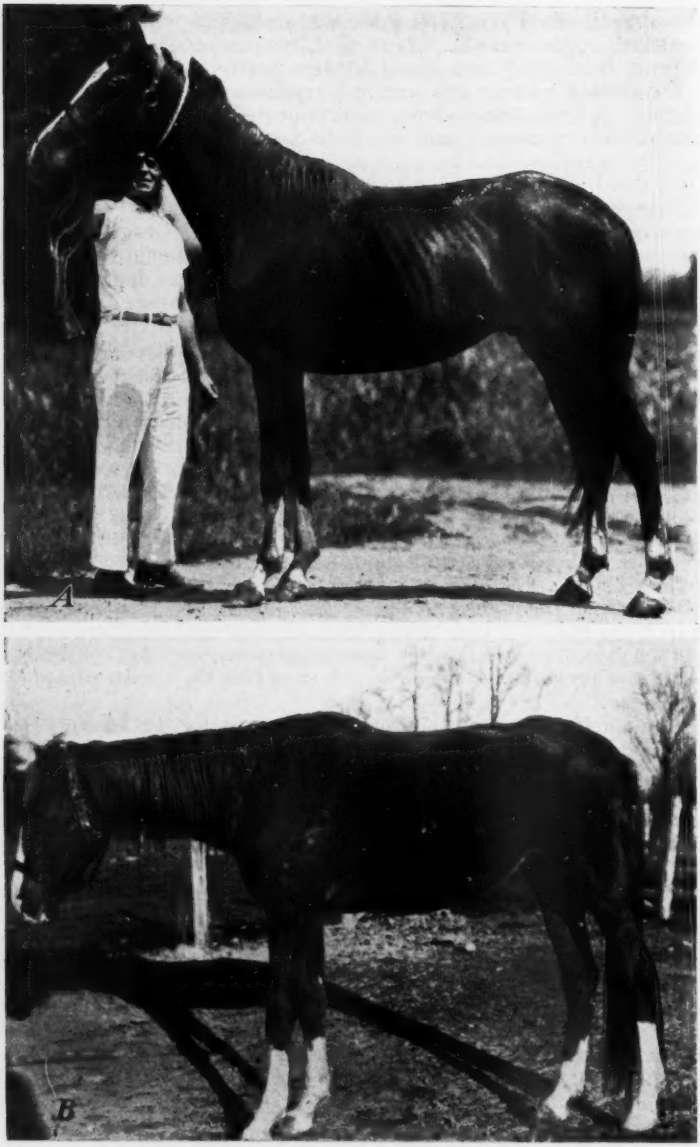

FIGURE 3.—*A*, Horse 980 as it appeared 1 week after the beginning of selenium feeding, and *B*, as it appeared 3 days before death occurred, or about 17 months after the beginning of the experiment. Note emaciation, drowsiness, and matted hair of mane in *B*.

was 17.3 parts per million of feed at the beginning of the experiment. In spite of the fact that the appetite was fairly good until a day or two before death, it will be seen in figure 1 that the animal's weight began to decrease within 2 weeks after selenium was added to the diet. The decline was gradual for about 2 months and then became much faster until death occurred. During the selenium-feeding period, there was a loss in weight of about 200 pounds.

Aside from emaciation, drowsiness, and some roughness of the horn at the top of the hoofs, relatively few symptoms were noted in this animal. Its condition 1 week after the experiment began is shown in figure 5, *A;* and *B* shows its appearance 4 months later, or 2½ months before death. At that time there had been a loss of about 100 pounds in weight. Changes in the horn at the top of the hoofs, manifested by a narrow white band particularly noticeable on the left front hoof, together with perceptible swelling of the coronary band, had just

FIGURE 4.—Front feet of horse 980 after 1 year from the beginning of the experiment. Note swelling of the coronary band, *a*, and the ring of roughened horn on the wall of the hoof below the hair line, *b*.

begun to appear. This was somewhat later than the initial appearance of such changes in the hoofs of the horses. Loosening of the hair, however, appeared in this animal at about the same time as in the horses, and at the time of death the hair in the mane was loose.

Post-mortem examination of the carcass showed it to be in very poor condition. The upper portion of each hoof had a slightly bulging appearance, and there was some roughening of the horny wall just below the coronary band. The pleural cavity contained about 300 to 400 cc. of clear, reddish yellow fluid. There were a few pneumonic areas in the posterior lobe of the left lung as well as in the anterior lobe of the right lung, which also showed some passive congestion. A small quantity of gelatinous fat was present around the pericardium and along the coronary groove of the heart. The pericardial sac was thickened, and a few petechiae were found on the epicardium. A

few small gray patches were found on the endocardium of the left ventricle and some small subendocardial hemorrhages in the right ventricle as well as on the mitral valve. When sectioned, the myocardium was chocolate colored. Aside from being slightly darker in

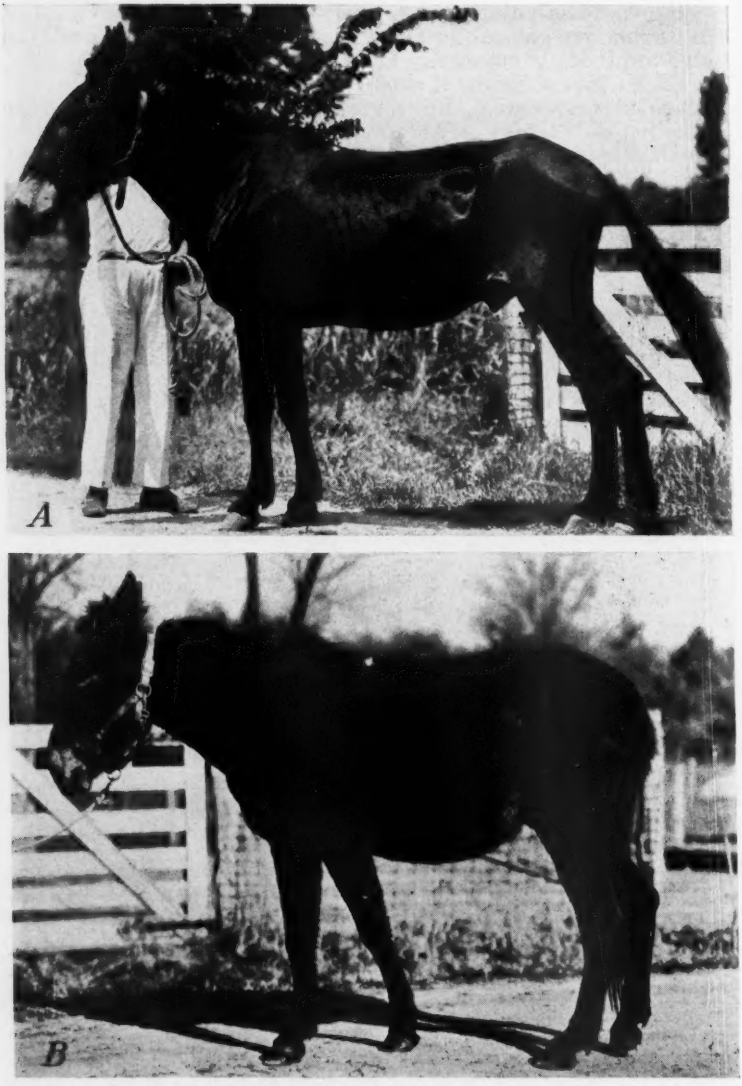

FIGURE 5.—*A*, Mule 1034 as it appeared 1 week after the beginning of selenium feeding, and *B*, after 4 months of selenium feeding and about 2½ months before death.

color, the liver appeared more or less normal. Nothing unusual was found in the spleen or the intestines. There was some nephritis in the kidneys, and the bladder, which was distended with slightly cloudy, yellowish urine, showed a mild catarrhal cystitis. The articular surfaces of both the hip and stifle joints were slightly pitted and roughened, and each joint contained a small quantity of thick stringy fluid.

HORSE 1023

Horse 1023 was included in the experiment to determine what effect the high level of selenium (96 p. p. m.) which the other two horses were receiving would have on a horse that had not previously been given selenium. The animal died 35 days after being placed on the selenium diet. The changes in the weight are shown in figure 1. Although the horse never ate all the feed given at any time and consequently did not get the full dose of selenium, there is no doubt that the loss of weight was largely due to the action of the selenium. About 4 days after the first dose was given, diarrhea appeared and looseness of the bowels persisted until the animal's death. A few hours before death, the horse manifested colicky symptoms and seemed to be in a great deal of pain. Few symptoms of chronic selenium poisoning, other than emaciation, were observed in this horse.

Post-mortem examination showed the carcass to be very thin. There was slight scaling of the horn just below the coronary band of all four hoofs. Some hypostatic congestion was found in the right lung, and there were subpleural hemorrhages on the right side. The fat of the heart was yellow and gelatinous, and a few small hemorrhages appeared in the fat along the coronary groove, as well as subepicardially on the left ventricle. Massive subendocardial hemorrhages were present in the left ventricle and petechiae in the right one. There was cirrhosis of the liver and the lobules stood out prominently. The spleen showed numerous small black petechiae under the capsule. Hemorrhages occurred in the fat around the kidneys, the capsules of which were removed with difficulty, and the kidney tissue itself was very friable. The cortex contained pin-point hemorrhages, and the medullary portion was reddened. In the mesentery, the fat tissue was yellow and gelatinous and large hemorrhages were sprinkled throughout. The stomach was found to be filled with oats, whereas the intestines were empty and slightly inflamed.

CHEMICAL DATA

The urine and feces were found to contain comparatively large quantities of selenium. These data are given in figures 6 and 7. The total quantity of excreta was not determined so that no estimation of the total quantity of selenium eliminated can be given. However, the high concentration in the excreta suggests that a large part of the ingested selenium was eliminated. From the analysis of portions of the horse carcasses and from analyses of small animals for selenium (7), it is believed that the average concentration for horses would be less than 1 part per million. Horse 962 weighed 660 pounds at death. If it is assumed that the carcass contained 1 p. p. m. of selenium, 0.3 gm. of approximately 250 gm. ingested would have been retained in the body of the animal.

There was an irregular but gradual increase in the selenium content of the blood while the horses were on the diet containing 24, and the mule 17.3, parts of selenium per million parts of feed (fig. 8). When the selenium content of the diet of the horses was increased to 48 p. p. m., the concentration in the blood became greater. The increase in the selenium content of the diet to 96 p. p. m. caused the concentration in the blood to continue to increase. At death the blood of horse 962 contained 9 p. p. m., and 3 days before death the blood of horse 980 contained 6 p. p. m. For both animals this was a sharp increase during the last week from a level of approximately 3.5 p. p. m. Horse 1023, which was begun on the high selenium diet, lived about 5 weeks and also showed a sharp increase in selenium concentration in the blood, ending with 9 p. p. m. at death. Mule 1034 died shortly after the

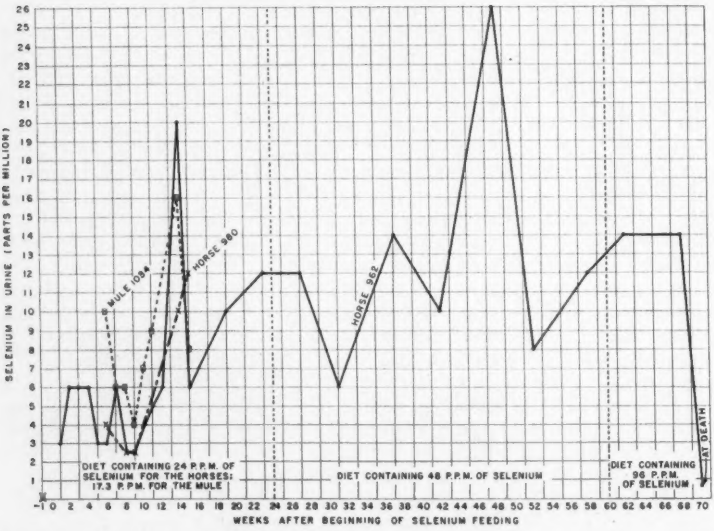
SELENIUM IN URINE (PARTS PER MILLION)
MULE 1034
HORSE 980
HORSE 962
AT DEATH
DIET CONTAINING 24 P.P.M. OF SELENIUM FOR THE HORSES; 17.3 P.P.M. FOR THE MULE
DIET CONTAINING 48 P.P.M. OF SELENIUM
DIET CONTAINING 96 P.P.M. OF SELENIUM
WEEKS AFTER BEGINNING OF SELENIUM FEEDING

FIGURE 6.—Selenium content of urine from equines on a diet containing sodium selenite.

change from 17.3 to 34.6 p. p. m., and during the 3 days before death the selenium concentration of the blood changed from 2 to 5 p. p. m.

In table 1 are given the analyses of various parts of the body of the horses and mule that died as a result of small daily doses of sodium selenite, together with data obtained on horses and a mule that died from a single dose of sodium selenite. The concentration of selenium in the blood of the animals that died as a result of daily doses of selenium is appreciably higher than in that of the animals that died from a single dose. The kidneys of those that died from a single does were higher in selenium than the kidneys of those that received daily doses. This finding was reversed in the case of the spleen, as is shown by data from the horses that survived more than a year (Nos. 962 and 980). The hoofs of the animals that received daily doses had an accumulation

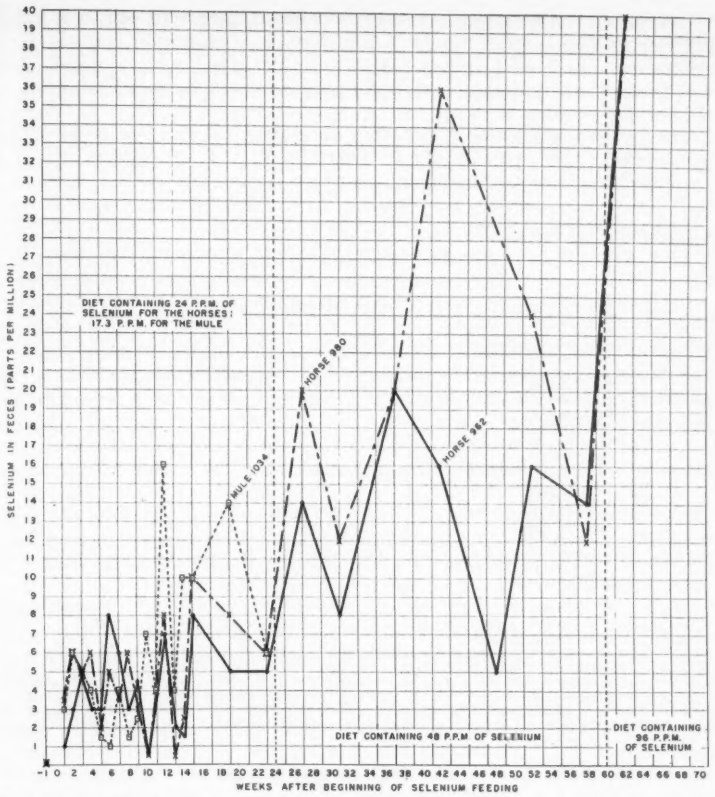
SELENIUM IN FECES (PARTS PER MILLION)
WEEKS AFTER BEGINNING OF SELENIUM FEEDING
DIET CONTAINING 24 P.P.M. OF SELENIUM FOR THE HORSES; 17.3 P.P.M. FOR THE MULE
MULE 1034
HORSE 980
HORSE 962
DIET CONTAINING 48 P.P.M. OF SELENIUM
DIET CONTAINING 96 P.P.M. OF SELENIUM

FIGURE 7.—Selenium content of feces from equines on a diet containing sodium selenite.

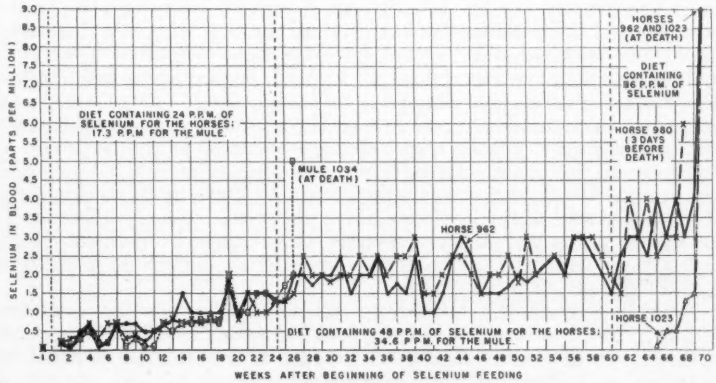
SELENIUM IN BLOOD (PARTS PER MILLION)
WEEKS AFTER BEGINNING OF SELENIUM FEEDING
DIET CONTAINING 24 P.P.M. OF SELENIUM FOR THE HORSES; 17.3 P.P.M. FOR THE MULE
MULE 1034 (AT DEATH)
HORSES 962 AND 1023 (AT DEATH)
DIET CONTAINING 96 P.P.M. OF SELENIUM
HORSE 980 (3 DAYS BEFORE DEATH)
HORSE 962
HORSE 1023
DIET CONTAINING 48 P.P.M. OF SELENIUM FOR THE HORSES; 34.6 P.P.M. FOR THE MULE

FIGURE 8.—Selenium content of blood from equines on a diet containing sodium selenite.

of selenium. The concentration became greatest in the old horny portion of the hoof. In the other portions examined, no clear-cut difference was found between the animals killed with a single dose and those that died as a result of repeated small doses of selenium.

TABLE 1.—*Selenium content of material from equines that died from repeated small doses of sodium selenite and from equines that died from a single dose*

Animal designation	Method of administering selenium	Selenium in—								
		Blood	Urine	Kidneys	Liver	Lungs	Spleen	Heart	Hair	Hoof
		P.p.m.	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*
Horse 1023	Daily doses of selenium for 5 weeks.	9.0	0.2	1.5	2.0	0.5	0.5	0.2	5.0	0.5
Mule 1034	Daily doses of selenium for 26 weeks.	5.0	8.0	2.0	1.5	2.0	.7	.3	-------	(1)
Horse 980	Daily doses of selenium for 68 weeks.	[2] 6.0	-------	5.0	2.5	2.5	3.5	.2	-------	(3)
Horse 962	Daily doses of selenium for 70 weeks.	9.0	.7	3.5	5.0	2.0	5.0	1.0	2.5	(4)
Horse 764 [5]	Single lethal dose of 4.6 mg. per pound of body weight.	2.0	-------	10.0	5.0	1.5	.4	.4	-------	.2
Horse 875 [5]	Single lethal dose of 3.65 mg. per pound of body weight.	2.5	-------	8.0	8.0	1.5	.5	.4	-------	.2
Horse 1009 [5]	Single lethal dose of 2.0 mg. per pound of body weight.	-------	1.4	10.0	2.0	.7	.5	.5	-------	-------
Mule 1035 [5]	Single lethal dose of 1.46 mg. per pound of body weight.	2.0	4.0	6.0	2.0	1.0	1.0	1.0	-------	-------

[1] Top one-third, 0.5 p. p. m.; middle one-third, 1.2 p. p. m.; bottom one-third, 1.2 p. p. m.
[2] Concentration 3 days before death.
[3] Inside soft material of top one-third, 0.3 p. p. m.; outside horny material of top one-third, 1.0 p. p. m.; inside soft material of middle one-third, 0.5 p. p. m.; outside horny material of middle one-third, 5.0 p. p. m; inside soft material of bottom one-third, 5.0 p. p. m.; outside horny material of bottom one-third, 8.0 p. p. m.
[4] Inside soft material of top one-third, 1.0 p. p. m.; outside horny material of top one-third, 5.0 p. p. m.; inside soft material of middle one-third, 1.0 p. p. m.; outside horny material of middle one-third, 2.0 p. p. m.; inside soft material of bottom one-third, 4.0 p. p. m.; outside horny material of bottom one-third, 10 p. p. m.
[5] By Miller and Williams (*4*).

DISCUSSION

From the results of this experiment, there appears to be little doubt that chronic poisoning can be induced in equines when repeated small doses of selenium are fed in the form of sodium selenite. Under the conditions of this experiment, however, all the characteristic symptoms of chronic selenium poisoning which have been described in animals maintained on naturally selenized feed (*2*, *5*) have not been reproduced, even during a prolonged period of feeding. Emaciation and drowsiness were marked in all four of the animals. In the two horses fed for the longest period, slight changes occurred in the character of the horn of the hoofs. In addition, the long hair of the mane and tail became loose, but extreme depilation or these parts or sloughing of the outer wall of the hoof was never observed. On the other hand, the lesions present at autopsy, namely, the changes in the heart, liver, and spleen, were similar to those reported as occurring in natural cases of the disease (*1*, *5*).

In this connection, it is of interest to contrast the effects, in equines and swine, of continued feeding of small doses of selenium as sodium selenite. In the latter species 50 percent of the animals exposed to selenium as sodium selenite manifested typical clinical symptoms

within 3 months (*3*), whereas in equines certain characteristic symptoms of chronic selenium poisoning failed to develop in 17 months. Some explanation for this difference may be found in the fact that the swine ate relatively little selenized feed after the first few days of the experiment, whereas no impairment of appetite was noted in the horses until immediately before death. This fact might account, therefore, for the increased resistance of the equines to the action of the selenium. When single large doses of selenium were given to equines and swine, however, the results were reversed (*4*). In that experiment it was found that the minimum lethal dose for horses was about 1.5 mg. per pound of body weight, whereas 6 mg. per pound of body weight only caused the swine to become sick.

In view of the results found in the present experiment and those involving single doses of selenium, it is believed that some tolerance to the action of selenium, at least in the inorganic form, may be acquired by some animals. The number of equines included in the present experiment is too small to permit a definite conclusion to be drawn. It should be pointed out, however, that about 6 months after selenium feeding began, horse 962 received daily 0.58 mg. of selenium per pound of body weight, horse 980 received 0.53 mg., and mule 1034 received 0.48 mg. The mule lived only 3 weeks at this level of feeding, but the two horses showed relatively little ill effect. About 15 months after the experiment began, the quantity was again increased, and the relation of selenium to body weight for horse 962 was 1.20 mg. daily per pound and for horse 980, 1.12 mg. Since the animals were losing weight regularly the quantity of selenium continued to increase in relation to body weight. When it is considered that a single toxic dose of the element has been found to be 1.5 mg. per pound of body weight for horses and that one horse (No. 980) survived for nearly 2 months and the other one (No. 962) lived 2½ months on daily doses almost as large, it would seem that these animals had built up some tolerance for selenium. This belief is further strengthened by the result of feeding about the same concentration of selenium to horse 1023. When this animal was placed on experiment, it received 1.19 mg. of selenium per day per pound of body weight and it survived but 1 month.

During the approximately 2 months that horses 962 and 980 lived after the selenium content of the diet was increased to 96 p. p. m., there was a gradual increase in the selenium content of the blood, marked by a sharp increase just prior to death. During the last 10 days the appetite decreased until the intake of selenium was relatively small. These facts suggest that there was a break-down in the elimination of selenium from the blood. The urine sample taken from horse 962 at death contained but 0.7 p. p. m. as compared with 6 to 26 p. p. m. during the whole previous year.

SUMMARY

To study the effect on equines of long-continued feeding of selenium in inorganic form, small daily doses of sodium selenite were administered in the feed to three horses and a mule. The animals were apparently in good health when the experiment began. The work

was carried on in 1937 and 1938 at the Animal Disease Station, Beltsville, Md.

At the beginning of the experiment a measured quantity of M/10 solution of sodium selenite was added to the oats at the rate of 24 parts of selenium per million of the total ration for two horses and 17.3 parts per million for the mule. The animals ate the selenized oats readily at first, but after about 2 months refused them and ate only hay. The sodium selenite solution was then given as a drench for about 4 months, when the quantity of selenium was doubled, making a level of 48 p. p. m. for the horses and 34.6 for the mule. About 4 months later, because of difficulty in drenching the horses, the mule having died in the meantime, the method of administering the sodium selenite was changed so that the solution was diluted and sprinkled on both the oats and hay. The level of selenium was raised to 96 p. p. m. of feed for the two horses about 15 months after the beginning of the experiment. A month later a third horse was placed on experiment and immediately given approximately 115 parts of selenium per million parts of feed daily.

The mule survived for only 6½ months after selenium feeding began, but one of the horses lived about 17 months and another 17½ months. The third horse, which received the highest level of selenium at the beginning of the experimental feeding, lived only about 5 weeks. The principal symptoms of chronic selenium poisoning exhibited by these animals were emaciation in spite of a fairly good appetite, listlessness, looseness of the hair of the mane and tail, and softening and scaling of the horny wall of the hoof. None of these symptoms, however, were so outstanding as those described in cases of chronic poisoning which occur under natural conditions. The lesions observed in the heart, liver, spleen, and kidneys at autopsy resembled those described in field cases, to a large extent.

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FEATHERING, GROWTH, FEED CONSUMPTION, AND RACHITOGENESIS IN CHICKS AS INFLUENCED BY THE KIND OF GRAIN IN THE DIET [1]

By H. L. WILCKE, *collaborator*, and JOHN C. HAMMOND, *associate biologist, Animal Nutrition Division, Bureau of Animal Industry, United States Department of Agriculture* [2]

INTRODUCTION

The cereal grains occupy a prominent position in the diets of poultry of all ages, yet definite information regarding the value of each of these cereals for specific purposes is lacking. Only within the last few years have there been active attempts to compare the cereal grains used with respect to their growth-promoting and bone-forming properties for chicks. The feathering of growing chicks is important to the broiler producer and to all producers of chicks because well-feathered broilers sell to much better advantage than others and because there is less danger of cannibalism among well-feathered chicks.

REVIEW OF LITERATURE

Since the literature has been reviewed in a recent article by Branion, Stackhouse, and Hull (*1*),[3] it is necessary only to add a few comments on data which have appeared since their paper was published. Branion and his coworkers (*1*) found no evidence of any anticalcifying property of oats or oat groats in comparison with corn, wheat, or barley.

The Council on Foods of the American Medical Association (*3*) published a report in which it was concluded that the experimental results available "may be explained on the basis of the calcium and phosphorus ratio in the diet together with a knowledge of the availability of the phosphorus," and further, "that there is not good evidence for the existence of a decalcifying factor in cereals." These conclusions are further substantiated by Mottram and Palmer (*9*), who found that the rickets produced by cereal diets was due to a low calcium content of the diets and that this condition could be corrected by the addition of calcium or of vitamin D. Rickets was not prevented by the addition of available phosphorus. These results do not agree with the explanation offered by Bruce and Callow (*2*), who suggested that the rachitic condition was caused by a lack of available phosphorus in the diets.

McDougall (*7*) found that the addition of 11 percent of lard or olive oil to the diet prevented rickets in rats which were fed diets low in calcium and vitamin D and made up largely of wheat flour or bread.

[1] Received for publication April 12, 1940.

[2] By authorization of the Bureau of Animal Industry, H. L. Wilcke, research professor of poultry husbandry, Iowa Agricultural Experiment Station, collaborated in conducting this work at the U. S. Department of Agriculture, Beltsville Research Center, Beltsville, Md. The authors are indebted to M. A. Jull, formerly of the U. S. Department of Agriculture, for arranging for the collaboration; to Harry W. Titus for his assistance, advice, and helpful criticisms; and to C. A. Denton and H. E. McClure for analyses of feed and bones.

[3] Italic numbers in parentheses refer to Literature Cited, p. 379.

The action of the fat or oil is based on the increased absorption of calcium due to the formation of calcium soaps.

Miller and Bearse (*8*) presented evidence to show that pullets fed a growing and laying ration with oats as the sole cereal developed less cannibalism than those fed a ration containing corn as the sole cereal. In addition, Kennard and Chamberlin (*6*) have published results indicating that oats have a definite value in the laying ration. The review by Crampton (*4*) of work comparing the various cereal grains for both chicks and laying birds demonstrates clearly the need for additional information regarding the relative value of the cereal grains in avian nutrition.

PURPOSE AND PLAN OF THE EXPERIMENT

The purpose of this experiment was to study the effect of oats, barley, corn, and wheat on feathering and growth, on the percentage of ash in the tibiae and their size, and on the feed consumption of chicks receiving diets with each of these grains as the principal cereal portion. Particular emphasis was placed on oats, since some of the earlier work had indicated a rachitogenic factor in this cereal when rats or puppies were the experimental animals. The experiment was conducted between May 15 and August 7, 1936, at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md.

Six hundred Single-Comb White Leghorn chicks were selected from a larger group which had been hatched from eggs from mass-mated pullets at the Beltsville Research Center. These chicks were hatched on May 15, 1936, and the following morning they were leg-banded by the use of duplicate bands. The chicks were weighed to one-tenth of a gram on an automatic scale, the weight of the bands being balanced with two bands on the scale. The chicks were then divided into 12 lots of 50 each, placed at random in the various lots. Each lot was kept in a separate colony brooder house in which sand was used as a litter, and the chicks were fed and watered immediately. None of them were allowed out of the houses at any time during the experiment. The lots were numbered from 412 to 423 to correspond to the diet numbers, and the chicks were weighed at biweekly intervals.

The plan of the experiment was to feed four lots of chicks all-mash diets made up of a single cereal grain (oats, barley, corn, or wheat) with dried buttermilk as the protein supplement, and sufficient high-grade ground limestone and chemically pure tricalcium phosphate to adjust the calcium and phosphorus to equal levels.

In order to determine the fraction of the grain in which the rachitogenic factor might be found, if it proved to be present, lots were included in which 20 percent of ground oat hulls supplemented corn and wheat. It was calculated that the oats diet contained approximately 20 percent of hulls, and this level was used to equalize the quantity of hulls in these diets with that in the oats diet. In addition, lots were included in which 20 percent of ground hulled oats supplemented the corn and the wheat, and also lots in which 20 percent of ground whole oats supplemented the corn, wheat, and barley. Finally, one lot on a standard mixed diet was included in the experiment as a control lot.

All the diets except the control were made up to contain as nearly as possible 16.6 percent of protein (dried buttermilk was used as the only

protein supplement in order to keep this part of the diet uniform), 0.75 percent of calcium, and 0.54 percent of phosphorus. In addition, 1 percent each of salt (sodium chloride) and cod-liver oil was used.

TABLE 1.—*Composition of the experimental diets except the control*

Ingredient	Parts, by weight, of diet No.—										
	412	413	414	415	416	417	418	419	420	421	422
Ground whole oats	77								20	20	20
Ground yellow corn			66¾				42½	52	49¼		
Ground barley		74									54½
Ground wheat				77	49¼	59⅞				57	
Ground hulled oats						20		20			
Ground oat hulls					20		20				
Dried buttermilk	21	24	31½	21	29	18	36	26	29	21	23½
Ground limestone	1	1	½	1	¾	1	¼	¾	⅛	1	1
Calcium phosphate			¼		⅛	⅛	¼	¼	⅝		
Salt	1	1	1	1	1	1	1	1	1	1	1
Cod-liver oil	1	1	1	1	1	1	1	1	1	1	1
Total	101	101	101	101	101	101	101	101	101	101	101

It was not possible to analyze the ingredients before the diets were made up, but samples of each ingredient were taken as the diets were mixed, and analyses were obtained later. One hundred pounds of each diet was mixed without cod-liver oil. The oil was incorporated in the feed at least once every 2 weeks. Only as much feed was mixed as would be used during that period. Table 1 shows the composition of all the diets except the control diet. The composition of the control diet (No. 423) was as follows:

	Parts by weight
Ground yellow corn	25
Wheat middlings	25
Rolled oats	10
Dried skim milk	10
Corn gluten meal	10
Alfalfa-leaf meal	8
Wheat bran	5
Yeast cells	3
Ground limestone	1. 5
Salt	1. 0
Cod-liver oil	1. 5
Total	100

When the chicks were 4 weeks of age, they were classified as to feather development, those which were completely feathered on the dorsal region being scored as group 1, those partly feathered as group 2, and those with only down on the backs as group 3.

Growth was measured by increase in weight at biweekly intervals for 12 weeks and also by three measurements made on the tibiae of five representative males and five representative females at 8 and 12 weeks of age. These measurements were (1) the length of shaft, taken with the epiphyseal cartilages removed, (2) the minimum diameter of the shaft at its midpoint, and (3) the maximum transverse diameter of the proximal head. After the bone measurements had been taken on the tibiae from the 8-week-old chicks, these bones were extracted and ashed according to the method described by Harshaw, Fritz, and Titus (*5*).

The feed was weighed for each lot at biweekly intervals, feed weights being taken on the same day as the chick weights. Feed consumption per chick per day and the total quantity consumed per chick for the entire period were calculated.

EXPERIMENTAL RESULTS

FEATHERING

The results of the classification of the chicks into feather groups and the number of males and females in each of these groups are presented in table 2.

TABLE 2.—*Number of chicks at 4 weeks of age in each of three feather groups as affected by the grain in their diet*

Lot No.	Kind of grain in diet	4-week-old chicks in—			
		Group 1	Group 2	Group 3	All groups
		Number	*Number*	*Number*	*Number*
412	Oats	45	3	1	49
413	Barley	36	13	1	50
414	Corn	1	10	37	48
415	Wheat	7	27	15	49
416	Wheat and oat hulls	4	31	15	50
417	Wheat and hulled oats	0	25	25	50
418	Corn and oat hulls	0	23	27	50
419	Corn and hulled oats	8	24	15	47
420	Corn and oats	0	13	34	47
421	Wheat and oats	9	28	12	49
422	Barley and oats	26	21	2	49
423	Mixed (control)	2	23	25	50
Total		138	241	209	588
Males		61	125	108	294
Females		77	116	101	294

It is obvious that the chicks in the various lots differed in respect to feather development. The question arose immediately as to whether the better feathering was due to the diet or whether it was associated with superior general development. In order to investigate this point, the chicks were grouped by sex into the three feather groups regardless of the diet which they had been fed. Their weights were analyzed by means of analysis of variance, the method of Snedecor (*10*) being used. Differences due to a disproportionate distribution of the sexes within the 12 lots were thereby eliminated.

A highly significant difference was found between the weights of the males and females. It is rather unusual to find a pronounced difference in the weights of the sexes at only 4 weeks of age; nevertheless, such differences were found for all the chicks in this experiment.

There were also highly significant differences among the weights for the three feather groups. Within each sex, the chicks which feathered most rapidly and, consequently, were classed as group 1 in feathering, were also the largest chicks. The data indicate that those diets which promote most rapid growth produce chicks that are most completely feathered at 4 weeks of age. This result is probably associated with rapid general development rather than with the diet. However, there was a distinct difference in the quality of the feathering of the various groups. The oat-fed chicks had a much superior quality of feather, whereas the corn-fed chicks were rated lowest in

this respect. The feather structure appeared to be modified slightly by the diet. The corn-fed chicks were rough in appearance, and many of them developed feathers which curled forward, presenting a frizzled appearance. The barbs, barbules, and barbicels all seemed to be present, but the barbicels did not appear to function normally in that the web of the feather was not smooth. This feathering is in marked contrast to that of the oat-fed group, which developed much smoother feathers. The addition of whole oats, oat hulls, or hulled oats improved the feathering of the chicks fed corn. This finding is direct evidence that there is some substance, factor, or group of substances or factors in oats which exerts a beneficial effect on growth and on the quality of feathering, but it was not possible to determine them from the results of this experiment.

The relationship between sex and feathering is not so pronounced. The interaction between these two characters was significant, but not

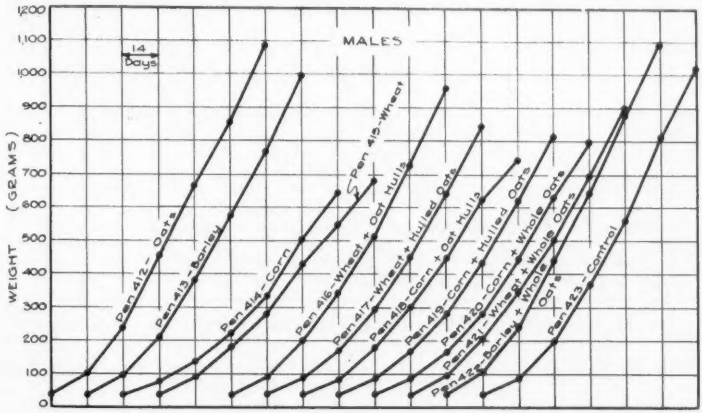

FIGURE 1.—Mean weights of males at biweekly intervals for 12 weeks.

highly so, indicating that there is probably a slight advantage in favor of the females in rate of feathering.

GROWTH AS MEASURED BY LIVE WEIGHT

The weights of the males and the females in the 12 lots are presented in figures 1 and 2. Since bone-ash determinations and bone measurements were made at 8 and at 12 weeks, the weights were also analyzed for differences at these periods, and, in addition, at the 10-week period, in order that these results might be compared with previous results in which analyses of data had been made only for the weight at 10 weeks.

The results of the statistical analysis were essentially the same for the three periods selected. There were highly significant differences in the weights of the chicks in the different lots at 8, 10, and 12 weeks. These results indicate that the diets affected the rate of growth of these chicks. However, in order to compare the individual diets, a more detailed analysis was necessary, and for this purpose the method suggested by Snedecor (*10*) was used to find the least mean difference which would be significant or highly significant.

The least differences which would be significant for the 8-, 10-, and 12-week data, respectively, were 35.67, 54.46, and 64.09 gm., and the least differences which would be highly significant were 46.96, 71.69, and 84.37 gm. Since the number of birds per lot varied only from 47 to 50, an arbitrary value of 48 chicks per lot was used in calculating these significant values for the 8-week data, and since 10 chicks had been removed from each lot at the end of the eighth week, the number per lot used for the 10- and 12-week data was 38.

An examination of the weight means for both males and females as presented in table 3 reveals the following significant differences:

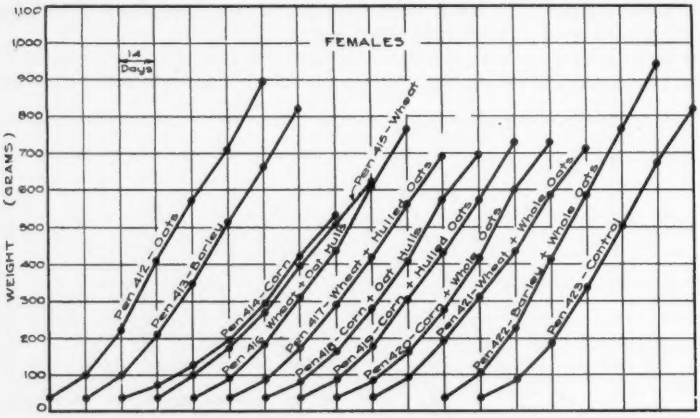

FIGURE 2.—Mean weights of females at biweekly intervals for 12 weeks.

TABLE 3.—*Average combined weights of male and female chicks in the 12 lots as affected by the grain in their diet*

Lot No.	Kind of grain in diet	Live weight at—			Lot No.	Kind of grain in diet	Live weight at—		
		8 weeks	10 weeks	12 weeks			8 weeks	10 weeks	12 weeks
		Grams	*Grams*	*Grams*			*Grams*	*Grams*	*Grams*
412	Oats	620	781	990	418	Corn and oat hulls	432	595	716
413	Barley	545	716	913					
414	Corn	317	469	594	419	Corn and hulled oats	433	598	771
415	Wheat	415	530	650	420	Corn and oats	429	612	758
416	Wheat and oat hulls	484	682	883	421	Wheat and oats	462	630	784
417	Wheat and hulled oats	435	597	760	422	Barley and oats	620	830	1,030
					423	Mixed (control)	534	744	921

The corn-fed chicks, lot 414, were definitely smaller than those in any other group.

The wheat-fed chicks, lot 415, were heavier than the corn-fed chicks but weighed less than those in any other group.

The chicks fed oats and those fed barley and oats, lots 412 and 422, were definitely larger than those in any of the other groups.

The addition of ground oat hulls to wheat increased the weight of the chicks more than did the addition of an equal quantity of either ground hulled oats or ground oats.

The addition of 20 percent of ground oat hulls, ground hulled oats, or ground oats to corn increased the weight of the chicks over that of those fed corn as the sole grain, and the differences in weight of the chicks fed these three supplements were not significant.

The differences in weight ranked in essentially the same order at 8, 10, and 12 weeks.

These results do not appear to be in complete agreement with those of Branion and his coworkers (*1*). However, the diets were not entirely comparable, since in the present work there were no more than two cereals in any one diet and only one source of protein was used.

There was a highly significant difference in the weights of the males and females at 8, 10, and 12 weeks, but this was to be expected. The interaction between sex and diets was not significant, indicating that the males and females reacted similarly to the diets fed.

GROWTH AS DETERMINED BY MEASUREMENTS OF TIBIAE

The mean measurements of the tibiae of the males and females at 8 weeks and at 12 weeks are presented in table 4.

TABLE 4.—*Mean measurements of the tibiae of the male and female chicks in the various lots at 8 and 12 weeks of age as affected by the grain in their diet*

MEASUREMENTS FOR MALES

Lot No.	Kind of grain in diet	At 8 weeks of age			At 12 weeks of age		
		Length of shaft	Diameter of shaft	Diameter of proximal head	Length of shaft	Diameter of shaft	Diameter of proximal head
		Cm.	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*
412	Oats	3.28	0.165	0.648	4.13	0.203	0.811
413	Barley	2.89	.160	.633	4.09	.196	.766
414	Corn	2.61	.127	.498	3.85	.184	.740
415	Wheat	2.95	.150	.562	3.77	.187	.708
416	Wheat and oat hulls	3.00	.144	.567	4.02	.195	.781
417	Wheat and hulled oats	2.95	.149	.546	3.80	.192	.741
418	Corn and oat hulls	2.93	.143	.562	3.86	.191	.754
419	Corn and hulled oats	2.88	.143	.549	3.95	.203	.772
420	Corn and oats	2.91	.143	.574	3.96	.195	.754
421	Wheat and oats	3.02	.147	.564	3.89	.184	.774
422	Barley and oats	3.30	.168	.628	4.26	.210	.828
423	Mixed (control)	2.95	.147	.517	4.20	.201	.806

MEASUREMENTS FOR FEMALES

412	Oats	3.10	0.159	0.592	4.06	0.206	0.728
413	Barley	3.29	.157	.574	4.01	.201	.698
414	Corn	2.43	.131	.485	3.61	.186	.644
415	Wheat	2.87	.152	.531	3.84	.190	.676
416	Wheat and oat hulls	2.80	.139	.514	3.93	.201	.713
417	Wheat and hulled oats	2.87	.140	.552	3.72	.187	.668
418	Corn and oat hulls	2.70	.138	.571	3.72	.186	.674
419	Corn and hulled oats	3.03	.142	.551	3.81	.191	.682
420	Corn and oats	2.75	.137	.520	3.91	.195	.718
421	Wheat and oats	2.74	.136	.524	3.72	.176	.647
422	Barley and oats	3.19	.168	.594	4.16	.202	.723
423	Mixed (control)	3.11	.145	.576	4.07	.202	.701

When these data were analyzed by the method of Yates (*11*) to adjust for missing data, it was found that the differences between lots were significant for all three measurements—length of shaft, diameter of shaft, and diameter of proximal head—at both 8 and 12 weeks of age. In addition, there were highly significant differences

between the sexes in length of shaft and diameter of the proximal head of the tibiae but not in diameter of the shaft. In general, the males had the longer bones and also the larger diameter of the proximal head, as would be expected because of their greater size and weight, but this relationship did not hold for the diameter of the shafts of these bones. The interaction between sex and diet was not significant, indicating that the sexes responded similarly to the various diets.

These results indicate that undoubtedly there are differences in the bone measurements for the various lots of chicks, but here again the question arises as to whether these differences are due to the diet or to general development. In order to clarify this point somewhat, the method of analysis which was used in connection with the weights was applied to these data. The least mean differences found to be significant and highly significant were as follows:

(1) For the diameter of the proximal head, the least differences that are significant and highly significant are approximately the same for both the males and the females at both 8 and 12 weeks, namely, 0.054 and 0.081 cm., respectively. (2) For the length of the shaft, the least differences that are significant and highly significant are 0.33 and 0.50 cm., respectively, for the males at 8 weeks, 0.42 and 0.61 cm., respectively, for the females at 8 weeks, and 0.71 and 1.03 cm., respectively, for both the males and the females at 12 weeks. (3) For the diameter of the shaft, the least differences that are significant and highly significant are 0.018 and 0.027 cm., repectively, for the males at 8 weeks, 0.005 and 0.007 cm., respectively, for the males and the females at 8 weeks, and 0.006 and 0.009 cm., respectively, for both the males and the females at 12 weeks. In making these calculations the same values were used for both sexes only when the variance was practically the same.

When the means of the various lots were compared, the above-mentioned measures for significant and highly significant differences being used, it was found that at 8 weeks the males on the corn diet had much shorter tibiae than those on several of the other diets. Significant differences also existed between other lots of males and between three lots of females. These results seemed open to question, and the data were reanalyzed by the use of the cube root of live weight instead of live weight itself. When the effect of the cube root of live weight was removed in this manner, no significant differences were found between the means for length of tibiae of either males or females at 8 or 12 weeks of age. Likewise, with the same procedure no significant differences were found between the means for diameter of proximal heads or diameter of the shaft of the tibiae in either sex at 8 or 12 weeks of age.

BONE ASH

The mean percentage of bone ash of the tibiae, with epiphyseal cartilages removed, for the males and the females are tabulated in table 5. These data were analyzed by analysis of variance, the method of Yates (*11*) being used to adjust for missing data.

The differences in percentage of bone ash of the males and females were not significant. This result is in agreement with those published by Harshaw, Fritz, and Titus (*5*), whose method of removing the epiphyseal cartilages was followed, and by whom it was pointed

out that sex differences appeared only when the percentage of ash was determined with bones on which the epiphyseal cartliages had been retained.

TABLE 5.—*Percentage of bone ash in tibiae of male and female chicks in the various lots at 8 weeks of age as affected by the grain in their diet*

Lot No.	Kind of grain in diet	Bone ash in tibiae of—			Lot No.	Kind of grain in diet	Bone ash in tibiae of—		
		Males	Females	Both sexes			Males	Females	Both sexes
		Percent	*Percent*	*Percent*			*Percent*	*Percent*	*Percent*
412	Oats	55.14	55.96	55.55	418	Corn and oat hulls	57.73	57.41	57.57
413	Barley	55.83	57.61	56.72	419	Corn and hulled oats	57.95	57.69	57.82
414	Corn	56.62	57.23	56.93					
415	Wheat	57.57	57.19	57.38	420	Corn and oats	57.45	57.98	57.71
416	Wheat and oat hulls	56.92	56.65	56.79	421	Wheat and oats	56.61	56.65	56.63
					422	Barley and oats	56.91	56.16	56.53
417	Wheat and hulled oats	58.70	58.78	58.74	423	Mixed (control)	57.65	56.85	57.25

There were highly significant differences in the bone ash among the various lots. When the individual lots were compared by combining the figures for males and females, it was found that the bone ash of the chicks fed the oats diet was significantly lower than that of all other lots. All the lots fed corn were definitely higher in bone ash than those fed oats. The differences among the remaining lots were not highly significant, except the lot fed wheat and hulled oats, which was the highest of any of the lots. The high ash content of the tibiae of the chicks in this lot can be accounted for only on the basis of the hulled oats, since lot 419, fed corn and hulled oats, the only other diet in which hulled oats was used, is not significantly lower, whereas lots 415, 416, and 421, in which wheat was fed, are all highly significantly lower. However, since only two lots were fed hulled oats and, furthermore, since this feed constituted only 20 percent of the diet of each, no definite conclusions can be drawn. The two lots fed diets in which barley appeared were among the lowest in percentage of bone ash, being significantly lower than six of the other lots. However, the biological importance of the low bone ash in the barley-fed lots may be questionable, since all values were well above the level usually considered as normal.

FEED CONSUMPTION

The data on feed consumption are presented in table 6. It is obvious that there were differences in the quantity of feed consumed per chick per day in the various lots, but as has been shown, there were also differences in the growth of the chicks, and these differences were in the same direction. That is, those lots of chicks which made the greatest gains in weight also consumed the most feed. Consequently, these data were subjected to analysis of covariance by the method of Snedecor (*10*). This analysis indicated highly significant differences among the various lots in feed consumption. However, the differences among the adjusted means for feed consumption were not significant, indicating that the various lots utilized the feed almost equally efficiently. Consequently, the differences in weight were a direct result of increase in feed consumption rather than in efficiency of the feed consumed.

TABLE 6.—*Feed consumption per chick in the various lots fed different grains*

Lot No.	Kind of grain in diet	Feed consumption per chick in 2-week period ending—						Total feed consumption per chick
		May 29	June 12	June 26	July 10	July 24	Aug. 7	
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
412	Oats	214.2	350.0	707.0	894.6	999.6	1,041.6	4,207.0
413	Barley	208.6	284.2	607.6	711.2	819.0	1,038.8	3,669.4
414	Corn	197.4	166.6	338.8	574.0	855.4	901.6	3,033.8
415	Wheat	207.2	214.2	455.0	574.0	856.8	884.8	3,192.0
416	Wheat and oat hulls	203.0	312.2	558.6	691.6	961.8	1,019.2	3,746.4
417	Wheat and hulled oats	214.2	203.2	362.6	543.2	924.0	882.0	3,129.0
418	Corn and oat hulls	198.8	229.6	499.8	702.8	845.6	942.2	3,418.8
419	Corn and hulled oats	176.4	197.4	385.0	631.4	859.6	1,006.6	3,256.4
420	Corn and oats	189.0	198.8	438.2	590.8	849.8	918.4	3,185.0
421	Wheat and oats	205.8	240.8	442.4	574.0	848.4	876.4	3,187.8
422	Barley and oats	214.2	326.2	572.6	813.4	1,003.8	1,008.0	3,938.2
423	Mixed (control)	190.4	252.0	483.0	729.4	953.4	950.6	3,558.8

DISCUSSION

It is a common opinion among poultrymen in general that corn is one of the most palatable grains used in poultry feeding. Nevertheless, in this work, the feed consumption of the chicks in the corn-fed lot was low throughout and the rate of growth of these chicks was correspondingly slow. The oats, barley, and oats and barley combination were utilized just as efficiently as the corn, and the addition to the corn of whole oats or of either the oat hulls or the hulled oats resulted in improvement of the corn diet. This was not expected in the case of the oat hulls, because there is a common supposition that an increase in the fiber content of a poultry diet, and particularly of a chick diet, will reduce the efficiency of that diet.

The only cases of cannibalism observed in this experiment were in the corn-fed lot of chicks. This finding is in agreement with results published by Miller and Bearse (*8*), who found that oats tend to prevent cannibalism in pullets.

At 4, 6, and 8 weeks of age, the chicks fed oats and those fed barley and oats were almost twice as large as those fed corn even though the former diets were fed at approximately the same level of protein, calcium, and phosphorus, and with a smaller supply of vitamins A and G complex than the corn diet.

The lower percentage of bone ash in the lots fed oats and barley than in the lot fed corn might be interpreted as indicating the presence of a rachitogenic factor in those two grains. However, the small reduction in bone ash caused by oats or barley at the high levels fed in these experiments would hardly be interpreted as of great biological importance, particularly when all ash values were at relatively high levels. In fact, it may be argued that it is equally undesirable to develop a bone ash that is excessively high, and in this case there is some question as to whether the oats and barley might carry a rachitogenic factor or whether corn may carry a factor for excessive calcification. In the results obtained here, neither hypothesis would be of biological significance because of the small differences obtained, but the results are interpreted as supporting the contention of Branion, Stackhouse, and Hull (*1*) that these cereals do not possess dissimilar properties in relation to calcification.

SUMMARY AND CONCLUSIONS

Twelve lots of Single-Comb White Leghorn chicks, each lot containing 50 birds, were fed oats, barley, corn, or wheat as the principal cereal portion of the diet. This work was carried on in 1936 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md.

Although the diet containing corn as the sole grain had larger quantities of vitamins A and the G complex than the other diets, the one with oats as the sole grain produced the most rapid gains of any of the diets containing single grains. The remaining single-grain diets ranked in the following order: Barley, wheat, and corn.

There appeared to be little difference in the efficiency of the diets used as measured by the units of feed necessary to produce a unit of gain in weight.

Chicks fed a diet in which corn was the sole grain had a very poor quality of feathering.

The addition of 20 percent of ground oat hulls, ground hulled oats, or ground whole oats to corn or to wheat diets improved the rate of growth and also improved the quality of feathering in the corn-fed chicks.

Oat hulls proved to be more effective than hulled oats or whole oats in supplementing wheat, but there seemed to be no difference in the value of these three products as supplements for corn.

The difference in bone development in the several lots was regarded as due to the effect of the diet on general development rather than to a specific effect on bone development.

Apparently oats and barley exert a slightly depressing effect on the percentage of ash in the tibiae of chicks, but this decrease in percentage of ash is slight and probably of little biological significance since the values for all lots were well above the minimum accepted as normal.

The data presented indicate that the four grains used do not possess rachitogenic properties.

Differences in rate of growth were due directly to differences in feed intake and not to differences in efficiency of the diets.

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THE EFFECT OF COOKING AND STORAGE ON THE ASCORBIC ACID CONTENT OF POTATOES[1]

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INTRODUCTION

Potatoes are consumed throughout a period beginning before their maturity and extending from 5 to 10 months after the tubers are harvested. During this period they undergo metabolic changes, the character and extent of which depend upon the duration and conditions of storage as well as upon the variety of potato. These changes are accompanied by changes in the ascorbic acid content. Since potatoes are never or rarely eaten raw, the effects of the numerous methods of cooking must also be considered if a true picture of their antiscorbutic value is to be obtained.

Although chemical methods of analysis simplify the quantitative determination of ascorbic acid, the application of these methods to biological material frequently creates new difficulties in the interpretation of results. Furthermore, in the case of the potato, insufficient information with regard to one or more of the common variables, time and temperature of storage, variety of potato, and cooking method, adds greatly to the difficulty of making a satisfactory comparison between the results of different investigators. Although the ascorbic acid content of raw potatoes is reported to vary between the wide limits of 53 mg. per 100 gm. (*10*)[3] and 1.5 mg. per 100 gm.(*13*), the results of most investigations indicate that newly harvested potatoes are highest in vitamin C value and that this quantity diminishes very rapidly during the first part of storage and later more gradually (*16, 18, 22, 26*). Kröner and Steinhoff (*8*) noted this decrease followed by a rise during the latter part of storage, and Pett (*18*) reported that sprouting caused an increase followed by a rapid decrease.

Fixsen (*4*) has summarized the data that show the effects of cooking upon the ascorbic acid content of potatoes. No general agreement exists. The same method of preparation reported by some investigators as causing a loss in ascorbic acid value, has been reported by others as apparently causing a gain; and one investigator reports that the same method of cooking results in a loss of ascorbic acid in one variety and a gain in another. Richardson, Davis, and Mayfield (*20*) reported a gain of 28 to 75 percent in ascorbic acid as a result of baking potatoes, whereas Lyons and Fellers (*12*) using the same method of cooking noted a loss of 50 percent. The gain in ascorbic acid found after cooking has been ascribed variously to the presence in the raw tuber of a protein-ascorbic acid ester capable of hydrolysis on cooking

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[3] Italic numbers in parentheses refer to Literature Cited, p. 393.

(*9*, *13*, *16*, *19*, 23), to the destruction of the ascorbic acid oxidase by heat (*3*, *14*), and to the increased permeability of cooked tissue to the extracting medium, which results in greater ease of extraction of the vitamin from cooked than from raw potatoes (*16*, *25*). It is hoped that the present investigation may point to definite conclusions on some of these controversial issues.

MATERIALS AND METHODS

SOURCE AND TREATMENT OF EXPERIMENTAL POTATOES

The Irish Cobbler and Green Mountain potatoes used in the major part of this study were furnished by the Bureau of Plant Industry and were grown at Aroostook Farm, Presque Isle, Maine. They were received on October 24, 1938, and placed in storage at 15.5° C. with a relative humidity of 80 percent. Six weeks later, half of the potatoes of each variety were removed to another storage room kept at 4.5° and a relative humidity of 80 percent. Sprouting was arrested at the storage temperature of 4.5° but continued in the potatoes stored at 15.5°. After a total of 4 months of storage, all sprouts were removed from the latter group. Any sprouts present were always removed at the time a potato was analyzed for ascorbic acid content.

The Irish Cobbler potatoes stored at 15.5° C. were used in a study of the effect of pressure cooking, baking, boiling (pared and unpared), and steaming (unpared) upon the ascorbic acid content of potatoes. For comparison, raw potatoes were analyzed simultaneously with potatoes cooked by each of these methods. These analyses of raw tubers, performed at intervals during the storage period, gave a history of the effect of storage at 15.5° upon the ascorbic acid content of Irish Cobbler potatoes. In addition determinations were carried out at intervals on the raw Green Mountain potatoes stored at 15.5° and on both varieties stored at 4.5°.

Since it was also desired to determine the effect of the so-called "waterless" cooking method upon the vitamin C value of potatoes, and there were not enough of the Maine-grown potatoes left in good condition, this part of the experiment was carried out on "new" Green Mountain potatoes obtained on the local market. Analyses were made of boiled pared potatoes and of pared potatoes cooked by the waterless method. Comparison was also made with raw potatoes simultaneously analyzed.

Summer Chippewa potatoes grown by the Bureau of Plant Industry at the experimental farm at Beltsville, Md., were made available for a study of the effect of maturity on the ascorbic acid content of potatoes. The analyses were begun 82 days after planting when the skins were still "feathery" and were made on the maturing tubers at semiweekly intervals for 6 weeks, by which time the vines were dead. The potatoes were delivered to the laboratory on the same day they were harvested and analyses were completed within 2 days. A disease, fusarium wilt, which ultimately became apparent in over half of these potatoes, developed at about the middle of the experimental period. Only tubers which appeared to be unaffected were analyzed. The effect of storage at 25.5° C. was also investigated.

SAMPLING PROCEDURE AND CHEMICAL METHODS

In the analysis of potatoes for their ascorbic acid content, the problem of representative sampling has often been overlooked or ignored. In the case of raw potatoes, grating or slicing and mixing, preliminary to sampling increases the possibility of formation of dehydroascorbic acid and of irreversibly oxidized ascorbic acid. However, unless the distribution of ascorbic acid is uniform throughout the tuber, there can be no assurance that a small single sample from a tuber is representative of the whole.

Preliminary experiments indicated that the distribution of ascorbic acid was not uniform throughout the tuber. All of the Maine-grown potatoes were sampled by the following method: Four wedge-shaped sections, weighing between 12 and 20 gm., were cut from the center to the outer edge from each potato. One of these sections was cut from the stem end, another from the bud end, and one from each side. The small size of many of the Chippewa potatoes made it possible to analyze entire tubers as one sample, and the larger potatoes of this group and of the Green Mountain potatoes bought on the local market were of such size that they could, in most cases, be represented accurately by two samples instead of four. When fewer than four samples were cut from a potato, care was taken that the wedges selected for analysis should represent both the stem and bud end of the tuber.

The ascorbic acid in raw potatoes was extracted by grinding each sample with 12 gm. of acid-washed sand under three successive portions (20 to 30 ml. each) of an aqueous solution containing 8 percent of acetic and 2 of metaphosphoric acid (*6*, *11*, *14*, *15*). The three extracts, separated from the pulp by centrifugation, were combined and made up to 100 ml. Two 25-ml. aliquots of each were titrated with 2-6-dichlorophenol-indophenol according to the method of Tillmans, Hirsch, and Hirsch (*24*) as modified by Bessey and King (*2*). All reagents were made up with glass-distilled water and all glassware was rinsed with glass-distilled water before use.

The average of the ascorbic acid content of all the samples of a single potato was taken as its ascorbic acid value and from 5 to 10 potatoes were used in each series of determinations. The analyses of raw potatoes were made on pared tubers whenever paring was introduced preliminary to the cooking method; in all other cases analyses were on unpared tubers.

Cooking causes a gelatinization of the starch in potatoes, and after the cooked tissue is ground with acid and sand, centrifugation yields a very cloudy suspension. It was found, however, that breaking up the potato tissue with a stirring rod and mixing it thoroughly with the acid resulted in the removal of essentially the same percentage of the total ascorbic acid with each extraction as when grinding with sand was employed (table 1). Also the resulting extracts were clearer and the end points at titration easier to distinguish. Only a fraction of the total ascorbic acid was removed by each extraction, and further experiments showed that with the weight of sample and size of centrifuge tube (50 ml.) used, 12 washings were necessary to remove all of the ascorbic acid from cooked potato tissue. However, six extractions constantly removed very close to 90 percent of the ascorbic acid present and at the same time the volume could be confined to 100 ml. As shown in table 1, this difficulty of removal of ascorbic acid was not encountered when raw potatoes were analyzed.

TABLE 1.—*Extraction of ascorbic acid from raw and cooked potato tissue*

Extractions (number)	Proportion of total ascorbic acid removed at end of each extraction					
	Cooked tubers,[1] samples—				Raw tubers, samples—	
	A	B	C	D	E	F
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	24.2	24.2	27.8	26.9	82.3	80.3
2	49.3	53.4	56.5	55.8	95.4	95.3
3	65.6	70.9	69.7	69.4	99.2	99.0
4	76.3	81.7	79.4	78.2	100.0	100.0
5	83.1	88.1	87.2	85.2	.....	.....
6	90.5	93.5	93.7	92.4	.....	.....
7	94.0	96.4	96.5	96.4	.....	.....
8	97.2	98.4	98.4	98.4	.....	.....
9	[2] 100.0	[2] 100.0	[2] 100.0	[2] 100.0	.....	.....

[1] Samples A and B were ground with sand in the second, third, and fourth extractions. The remaining extractions of these samples and all extractions of samples C and D were made without sand as described in the text.

[2] The titration value for the ninth extraction was so small that for practical purposes 100-percent extraction was assumed.

The following procedure was employed in the analysis of cooked potatoes: Centrifuge tubes containing about 20-ml. portions of the extracting medium were weighed and chilled in an ice bath. Four wedge-shaped samples were taken as soon as possible after the potatoes were cooked and each sample was cut immediately into small pieces and dropped into the cold acid. The tubes were then reweighed to determine the weight of the sample. Extraction was carried out with six successive portions of the mixture of acids, the potato tissue being thoroughly broken up and mixed with each fresh portion of acid. Centrifugation for 4 minutes at high speed gave a fairly clean separation of liquid and solid. The combined extracts were made up to 100 ml. and two aliquots of each sample were titrated.

In consideration of the results presented in table 1, the values for ascorbic acid so obtained were taken to be 90 percent of that present in the samples and the total amount was accordingly estimated on this basis. Finally, taking into account the loss or gain in weight on cooking, the values were readjusted to the basis of the raw weight of the potato.

COMBINED ASCORBIC ACID

McHenry and Graham (*13*) and others have reported the presence of a protein ester or "bound" ascorbic acid in potatoes. This compound is said to be soluble in water and insoluble in the ordinary ascorbic acid extractants, but capable of hydrolysis on heating or on treatment with 1 percent hydrochloric acid to free the soluble ascorbic acid.

Since both Green Mountain and Irish Cobbler potatoes are reported (*19*) to contain large quantities of this ester, various attempts were made to determine its presence. The residues remaining after the usual acid extraction of the raw potato tissue were treated with 1 percent hydrochloric acid according to Reedman and McHenry's directions (*19*). Several variations in time and temperature were used. In no case was there any additional titration value as a result of this treatment. The residue remaining after thorough extraction

of the potato tissue with trichloroacetic acid—a better protein precipitant than the acetic-metaphosphoric acid mixture—gave the same negative reaction to hydrolysis. This agrees with the results reported by Bessey (*1*) of similar experiments on Green Mountain and Irish Cobbler potatoes. Fujita and Ebihara (*5*) have reported recently that there are some grounds for believing the ascorbic acid in plant tissue to be partially bound to protein. They state, however, that this "bound" ascorbic acid is readily converted to the free form in the presence of metaphosphoric acid and is thus completely removed from the tissue by ordinary extraction procedure.

DEHYDROASCORBIC ACID

At various times during the course of the experiment, portions of the extract were treated with hydrogen sulfide for 20 to 30 minutes as a first step toward determining whether any dehydroascorbic acid was present. No increased reducing activity could be determined except in one case. Then it was not certain that all of the hydrogen sulfide had been removed. Bessey (*1*) found a small amount of dehydroascorbic acid in both Green Mountain and Irish Cobbler potatoes after storage. He recommends longer contact with hydrogen sulfide in a slightly less acid medium, which may account for the difference in his results.

EFFECT OF METHOD OF COOKING ON ASCORBIC ACID CONTENT OF POTATOES

In order that the potatoes cooked by different methods should attain the same stage of "doneness," cooking was discontinued when the internal temperature measured at the center of the tuber had reached 96° C. In each individual cooking, potatoes of approximately the same weight were used. A thermometer was inserted with the bulb at the center of one potato (two in baking) of each lot, with the exception of those prepared in the pressure saucepan and by "waterless" cooking. In the latter instances, the time required to bring a given weight of potato to the required temperature was determined by preliminary experiment, and this time was adhered to in the remainder of the tests. Potatoes without thermometers from each cooking lot were used for analysis. In the study of any given cooking method the tubers analyzed raw were of the same weight as those which were analyzed after cooking. The results of these analyses are given in table 2 in which the data showing the effect of each method of cooking upon the ascorbic acid content of potatoes are summarized.

BAKING

Three or four unpared Irish Cobbler potatoes weighing between 200 and 250 gm. were prepared in an oven maintained at a temperature of 215° C. The time required varied from 47 to 52 minutes. The potatoes lost 15 to 17 percent in weight and the average loss of ascorbic acid, calculated on the basis of raw weight, was 15 percent.

STEAMING

Three unpared Irish Cobbler potatoes weighing between 153 and 180 gm. were used for each cooking. They were cooked in a covered

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steamer over 4 quarts of rapidly boiling water. This method required from 33 to 39 minutes except in one case when the time extended to almost 51 minutes. There was usually a gain in weight on the order of 1 gm. per potato. No ascorbic acid was recovered in the water. About 10 percent of the ascorbic acid originally present in the raw potatoes was destroyed.

TABLE 2.—*Ascorbic acid losses in cooking Irish Cobbler and Green Mountain potatoes*

Variety and cooking method	Raw potatoes		Cooked potatoes			Ascorbic acid recovery in—		Ascorbic acid destruction
	Tubers analyzed	Ascorbic acid per 100 gm. [1]	Tubers analyzed	Ascorbic acid per 100 gm. (raw basis) [1]		Cooked potato	Cooking liquor	
				In potato	In cooking liquor			
Irish Cobbler, stored:	*Number*	*Milligrams*	*Number*	*Milligrams*	*Milligrams*	*Percent*	*Percent*	*Percent*
Baked	5	10.6±0.21	5	9.0±0.30	.....	85	.....	15
Steamed, unpared	10	10.1±0.19	8	9.1±0.14	.....	90	.....	10
Boiled, unpared	10	10.1±0.19	8	9.1±0.21	.....	90	.....	10
Steamed in pressure cooker, pared	6	9.7±0.15	6	8.2±0.21	0.4±0.03	85	4	11
Boiled, pared	8	10.3±0.12	8	7.8±0.20	0.5±0.05	76	5	19
Green Mountain, "new":								
Boiled, pared	10	20.0±1.34	10	16.1±0.92	2.3±0.29	80	12	[2] 8
"Waterless" cooking, pared	10	17.3±0.73	11	15.8±0.47	.....	91	.....	[2] 9

[1] The figures following the symbol ± represent the standard errors of the mean.
[2] The extent of destruction of ascorbic acid is not statistically significant.

BOILING

The potatoes were cooked by immersion in boiling, distilled water. The volume of liquid required to keep the potatoes completely covered in a 3-quart saucepan (7 inches in diameter) throughout the cooking period was calculated from the formula:

$$\text{Total weight of potatoes} \times 2.5 - 200 = \text{milliliters of water required.}$$

Potatoes were boiled pared and unpared. Three potatoes weighing between 140 and 180 gm. were used in each case. The unpared potatoes (Irish Cobblers) required from 33 to 40 minutes cooking time and gained about 1 gm. apiece in weight. They lost about 10 percent of their original content of ascorbic acid, none of which was found in the cooking liquid.

The pared potatoes required from 31 to 37 minutes for cooking. The Irish Cobbler potatoes gained from 2 to 6 gm. in weight and there was very little sloughing. The potatoes contained 76 percent of their original ascorbic acid and an additional 5 percent was recovered in the cooking liquid so that the destruction of the vitamin was 19 percent.

The "new" Green Mountain potatoes were more mealy than the Irish Cobblers, which had been stored, and there was a great deal more sloughing during cooking. Nevertheless these potatoes retained 80 percent of their ascorbic acid value. The cooking liquid contained another 12 percent. The average destruction of ascorbic acid in these potatoes was 8 percent, but because of the magnitude of the

standard error, this difference cannot be regarded as significant. If the ascorbic acid present in the cooking liquid were disregarded the loss would be 24 percent in the Irish Cobbler and 20 percent in the Green Mountain potatoes.

PRESSURE COOKING

For the pressure-cooking experiment a 3-quart pressure cooker made of cast aluminum was used. The cover, which was automatically steamtight, contained a vent pipe to which a weight was fitted so that pressure could be maintained at 15 pounds. It was possible to cool the whole cooker very rapidly by placing it in a pan of cold water so that cooking was stopped almost instantly when the utensil was removed from the flame.

Two Irish Cobbler potatoes weighing between 100 and 114 gm. were cooked at one time. They were placed on a rack in the cooker and one-fourth cup of hot water was added. Two minutes were required for the pressure to reach 15 pounds and it was maintained at this point for 15 minutes, giving a total cooking time of 17 minutes. The pressure was released in 10 to 15 seconds.

The gain in weight for different samples of potatoes varied from zero to 4.5 gm. They retained on an average 85 percent of their ascorbic acid value and an additional 4 percent was recovered in the small amount of liquid remaining at the end of the cooking period, so that the destruction of vitamin C was 11 percent, with a loss of 15 percent if this liquid was discarded.

"WATERLESS" COOKING

Four "new" Green Mountain potatoes weighing between 95 and 135 gm. were prepared at one time in a 1½-quart, heavy aluminum, covered pan. They were washed after paring, placed in the pan, rinsed in cold water, and the water poured off so that the potatoes remained moist. During the first half of the cooking period the temperature inside the pan was maintained slightly below the boiling point so that the formation of vapor was slow and its escape from the container not apparent. Thereafter the temperature was lowered and for the remainder of the cooking period it was maintained at 84° to 86° C. An indicator in the cover of the pan made control possible.

The total cooking period was 64 minutes. At the end of cooking there was no remaining liquid, the potatoes were very lightly browned at the points of contact with the pan, and there was a slight skin formation. They lost from 3 to 5 gm. apiece in weight and an average of 9 percent of their ascorbic acid content. In view of the magnitude of the standard error, this loss of ascorbic acid cannot be regarded as significant.

RELATION OF DEGREE OF MATURITY OF POTATOES TO ASCORBIC ACID CONTENT

The Chippewa potatoes analyzed semiweekly for 6 weeks prior to maturity decreased in ascorbic acid content during that period from 25.5 to 20.1 mg. per 100 gm. (table 3). The ascorbic acid content of the potatoes did not change between the twelfth and fourteenth week after planting. At the end of the fourteenth week a decrease in

ascorbic acid occurred simultaneously with the appearance of a diseased condition in the tubers. For this reason, although only apparently healthy tubers were analyzed, it is uncertain whether or not the decrease in ascorbic acid value is attributable to the maturity of the vines. Lyons and Fellers (*12*) found no loss of ascorbic acid in maturing Green Mountain potatoes.

There was a wide variation in the size of the potatoes throughout the entire experimental period. Tubers ranging in weight between 12 and 193 gm. were analyzed. As far as possible, the entire size range was represented in each lot of potatoes analyzed and no relationship was found between the concentration of ascorbic acid present and the weight of the tuber.

TABLE 3.—*The ascorbic acid content of maturing Chippewa potatoes per 100 gm. of tuber and effect thereon of short periods of storage*

Period after planting (weeks)	Without storage[1]		Stored at 25.5° C. for[1]—					
			1 week		2 weeks		3 weeks	
	Tubers examined	Ascorbic acid content	Tubers examined	Ascorbic acid content	Tubers examined	Ascorbic acid content	Tubers examined	Ascorbic acid content
	Number	*Milligrams*	*Number*	*Milligrams*	*Number*	*Milligrams*	*Number*	*Milligrams*
12	25	25.5±0.74						
13	10	26.4±1.15	12	20.6±0.47	7	19.2±1.50	10	17.0±0.76
14	13	25.9±1.05			7	17.8±0.69	10	17.0±0.76
15[2]	10	22.2±1.01					7	17.5±0.58
16	13	23.2±0.86			13	18.1±0.60		
17[3]	14	20.1±0.69	7	18.7±1.05				

[1] Figures following the ± symbol show the standard error.
[2] Fusarium wilt was well developed.
[3] The vines were dead at this time.

EFFECT OF STORAGE ON ASCORBIC ACID CONTENT OF POTATOES

Olliver (*16*) reported a loss of 50 percent of the ascorbic acid from new potatoes after 15 days of storage and Pett (*18*) a loss of over 50 percent after 20 to 30 days. In the present study there was also a marked decrease in the ascorbic acid content of stored potatoes, very rapid during the first few weeks after harvesting, and more gradual thereafter. The immature Chippewa potatoes lost 22 percent of their ascorbic acid after 1 week's storage at 25.5° C. and 36 percent in 3 weeks (table 3).

"New" Green Mountain potatoes bought on the market decreased in ascorbic acid content from 20.0 to 17.3 mg. per 100 gm., a loss of 14 percent in about 10 days at 25.5° C. These potatoes had probably been harvested for at least a week before the first analysis for ascorbic acid was made.

The Maine-grown Green Mountain potatoes lost about 30 percent of their ascorbic acid in the first month of storage at 15.5° C. (fig. 1).

Unfortunately no figures were obtained for the Irish Cobbler potatoes during the first period of storage, but the close agreement of the curve with that of the Green Mountain potatoes for the remainder of the 15.5° storage period suggests the probability of similar behavior at the beginning. Green Mountain potatoes stored at 15.5° for 5

months had an ascorbic acid content of 10.1 mg. per 100 gm., a loss of nearly 50 percent of their original value. Irish Cobblers stored for the same period and at the same temperature contained 9.9 mg. per 100 gm.

It is interesting to note that in these potatoes the rate of loss of ascorbic acid at 15.5° and 25.5° C. was apparently independent of the variety of the potato, but dependent, to some extent at least,

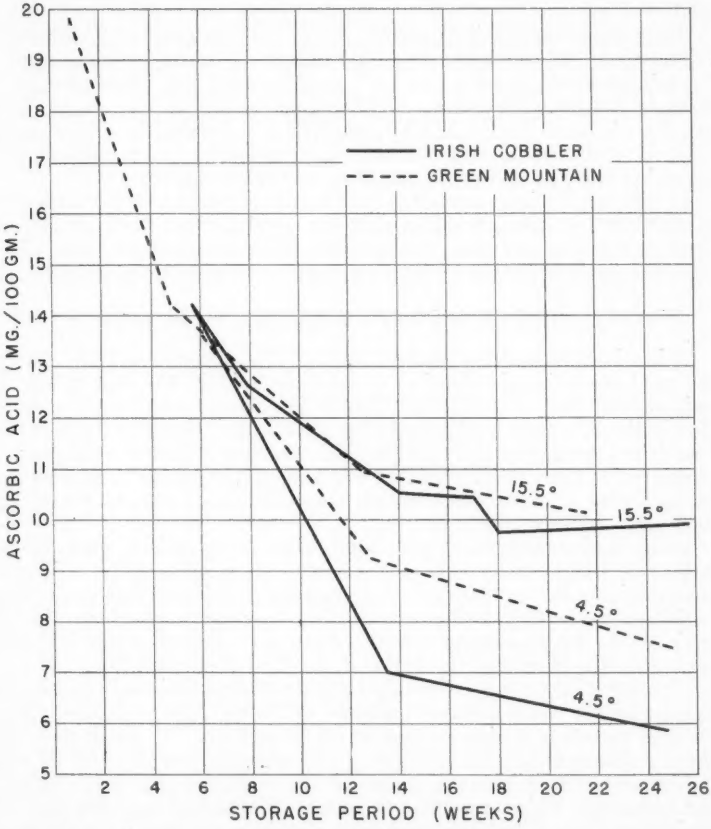

FIGURE 1.—Ascorbic acid content of Irish Cobbler and Green Mountain potatoes during storage at 15.5° and 4.5° C.

upon the ascorbic acid content of the tuber. The variation in ascorbic acid content from tuber to tuber was much smaller in potatoes that had been stored for a short period than in immature and new potatoes. This approach to uniformity during storage could only have been brought about if the loss of the vitamin were greater during the same period from tubers of high ascorbic acid content than from those of lower ascorbic acid content.

When Irish Cobbler and Green Mountain potatoes were placed in storage at 4.5° C. after a preliminary period of 6 weeks at 15.5° the loss of ascorbic acid was more rapid in both varieties as compared with the loss at 15.5°. At the end of the storage period (25 weeks) the ascorbic acid content of the Green Mountain potatoes had dropped to 7.5 mg. per 100 gm. and that of the Irish Cobblers to 5.9 mg. per 100 gm. Apparently the interference with the physiological processes caused by lowering the temperature has a marked effect upon ascorbic acid.

From these results it would seem that the storage of potatoes in cellars where the temperature approaches the freezing point during the winter would cause a greater loss of ascorbic acid than storage in warmer cellars. However, Kröner and Steinhoff (*8*), who kept potatoes in such a storehouse, found that the ascorbic acid content reached a low value and then increased steadily during the spring, following the rise in the temperature of their storehouse.

In view of the increase noted by these authors and of the data presented here, it seems possible that the detrimental effects of storage at a low temperature upon the ascorbic acid content of potatoes might be offset to some extent by later storage at a higher temperature.

DISTRIBUTION OF ASCORBIC ACID IN THE POTATO TUBER

It has been repeatedly stated (*7*, *12*, *17*) and apparently is usually assumed that the distribution of ascorbic acid in the raw potato is uniform or nearly so, although Rudra (*21*) found a higher concentration in the skin than in the flesh of new potatoes. Some of the potatoes used in the present study were examined after a period of storage, and it was found that, although there was a tendency for the concentration to be higher in the cortical layer, the difference between it and the medullary layer was slight.

When wedge-shaped samples were taken from various parts of the potato it was noticed that the concentration of ascorbic acid was always higher in the bud end of the tuber than in the stem end. This variation led to the method of sampling previously described, that of taking four wedge-shaped samples from each potato analyzed: One each from the stem and the bud end, and one from each side.

Figure 2 shows the relationship between the ascorbic acid content of these various wedge-shaped sections of Green Mountain and Irish Cobbler potatoes during storage at 15.5° and 4.5° C. Each point on the curves represents the average of the values of from 5 to 10 tubers.

A similar distribution was present in the cooked potatoes both pared and unpared. This shows that there was little diffusion of the vitamin during cooking. The sharp rise shown in the curve for the bud end of Irish Cobblers stored at 15.5° C. occurred just before the removal of all of the sprouts and the abrupt decline immediately after this treatment. No analyses were made of the Green Mountain potatoes at this time.

With regard to the potatoes studied later, the immature Chippewas showed practically no difference in the distribution of ascorbic acid in the various sections, although there was a slight tendency toward a higher concentration in the bud end as they approached maturity. The new Green Mountain potatoes bought on the market showed only a slight tendency to have a higher concentration of ascorbic acid in the

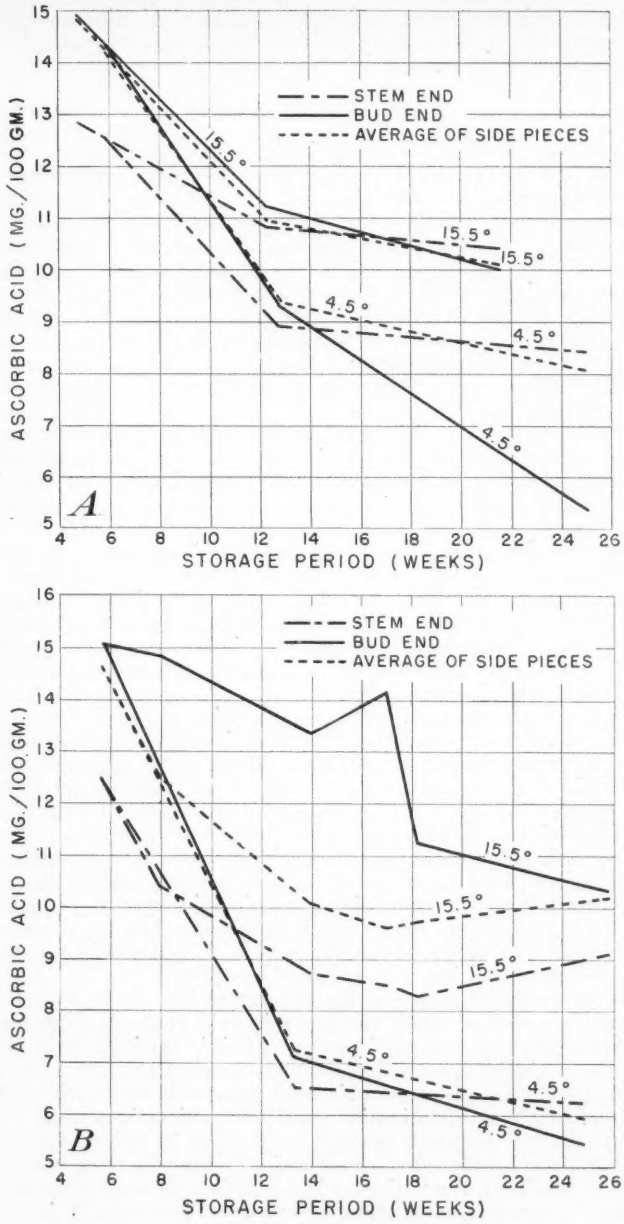

FIGURE 2.—Distribution of ascorbic acid in potatoes stored at 15.5° and 4.5° C.: *A*, Green Mountain; *B*, Irish Cobbler.

bud end than in the stem end when purchased. This difference became very apparent by the end of the first week and increasingly more pronounced thereafter.

DISCUSSION OF RESULTS

The results of the present study indicate that the vitamin C value of potatoes is more dependent upon the time that elapses after harvesting before the potatoes are consumed and the temperature at which they are stored during that time than upon the method of preparation for consumption. The method of cooking most commonly employed, the boiling of pared potatoes, causes the greatest loss in ascorbic acid content, particularly since the cooking liquor is usually discarded. Losses due to other methods of cooking are somewhat smaller and fairly comparable. However, the decrease in the ascorbic acid content of potatoes during the first few weeks after harvesting exceeds even that which would be produced by the most destructive of the cooking methods.

Data presented here in regard to the effect of cooking upon the ascorbic acid content of potatoes is in general agreement with that of Scheunert, Reschke, and Kohlemann (*22*) and Wachholder, Heidinger, Grieben, and Köhler (*26*). The former reported the effect on the ascorbic acid content of steaming unpared potatoes and the latter that of steaming unpared potatoes and of boiling both unpared and pared potatoes. Much greater losses in ascorbic acid than were found here have also been reported, while some investigators have noted similar losses with some of the methods of cooking and gains with others. No such gains were found in the present investigation and no evidence was obtained in support of any of the theories advanced to explain these gains.

The apparent inconsistencies of the gains reported for ascorbic acid in cooked potatoes suggest the operation of an accidental factor. If, for example, boiling pared potatoes causes an an apparent increase in ascorbic acid content, boiling unpared potatoes should have the same effect. However, if the type of distribution of ascorbic acid shown by Irish Cobbler potatoes at 15.5° C. were present but unrecognized, it is easy to see that the accidental selection of a disproportionately large number of samples from the bud end of the tubers would result in a relatively high ascorbic acid content as estimated from this group of samples. If, in addition, these samples represented cooked potatoes, the high value obtained would be thought to be a result of cooking rather than of sampling.

Another essential factor in the determination of the actual effect of a cooking method upon the ascorbic acid content of potatoes is the elimination of the effect of storage. This is particularly important when the potatoes are studied soon after being harvested since the rate of decrease in their ascorbic acid content is extremely rapid at this time. Unless determinations of the ascorbic acid content of both raw and cooked potatoes parallel each other in point of time, the result obtained for the effect of a cooking method may be very erroneous. In addition, the variation in ascorbic acid content from tuber to tuber is much greater soon after harvesting than it is after a period of storage. Consequently the sampling of an adequate number of tubers is imperative.

SUMMARY

In sampling potatoes for the determination of their ascorbic acid content, all sections of the tuber were represented in proportion to their presence in the potato. The vitamin was completely extracted from raw potato tissue more easily than from the cooked tissue. Six successive extractions removed almost a constant percentage of the total ascorbic acid from the latter and a factor was employed to calculate the total.

There was no evidence of the presence in potatoes of a "bound" ascorbic acid insoluble in the extracting medium employed. No dehydroascorbic acid was found in the potatoes analyzed.

New Green Mountain potatoes were used in a comparison of the effects of boiling and "waterless" cooking upon the ascorbic acid content of potatoes. The variation in the ascorbic acid content from tuber to tuber in the new potatoes was so great that the losses obtained as a result of cooking these potatoes could not be regarded as significant.

Irish Cobbler potatoes stored at 15.5° C. were used to study the effect of pressure cooking, baking, boiling, and steaming upon the ascorbic acid content. Steaming and boiling unpared potatoes were the most conserving of vitamin C. Baking and pressure cooking caused slightly larger losses of ascorbic acid, while boiling pared potatoes was least conserving of the vitamin. However, the maximum loss of ascorbic acid due to a cooking method never exceeded 25 percent.

Chippewa potatoes decreased in ascorbic acid content during maturation. Because of the development of fusarium wilt during the growing period it was uncertain whether the effect shown was caused by maturity or disease.

All three varieties of potatoes lost ascorbic acid during storage. At 15.5° C. the losses were most rapid during the first few weeks of storage and became more gradual thereafter until, at the end of 26 weeks of storage, the ascorbic acid content had nearly reached a plateau value. Storage of a lot of Green Mountain and Irish Cobbler potatoes at 4.5° caused their ascorbic acid content to drop below that of a similar lot kept at 15.5°.

Ascorbic acid was not distributed uniformly throughout the tuber. Moreover the relative distribution of ascorbic acid differed in Green Mountain and Irish Cobbler potatoes. It was affected by the temperature at which the tubers were stored and was subject to continuous change during the period of storage.

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STIMULATION OF GROWTH IN JUVENILE MANGOSTEEN PLANTS[1]

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INTRODUCTION

Much of the literature pertaining to the mangosteen, *Garcinia mangostana* L., laments the fact that this much-desired tropical fruit tree is so difficult to grow. The main difficulty lies in the oftenmentioned poor vigor of the young plants. In the nursery of the Puerto Rico Experiment Station of the United States Department of Agriculture at Mayaguez, there are some plants as much as 5 years of age which are no more than 6 inches high. Most plants fail to survive the juvenile stage, but the few that do survive develop to maturity in a manner much the same as seedlings of other tree species. The root system of a juvenile plant consists of a slender taproot having corky bark and little, often none, of the more active lateral system of smaller roots. In general, the poor development of mangosteen plants has been attributed to this poor root system.

Oliver,[2] working on the problem of mangosteen grafting under greenhouse conditions in Washington, D. C., about 1910, found that poor mangosteen plants could be approach-grafted on *Garcinia tinctoria*, a close relative. After such grafting, the mangosteen became vigorous, and new roots developed from the base of the mangosteen stem where the root system had been severed after union with the *G. tinctoria* stock had been accomplished. The new mangosteen root system continued to develop and ultimately the mangosteen reestablished itself upon its own roots and the *G. tinctoria* root system perished

Working with plants of *Aleurites fordii* whose growth had entirely ceased, Bonner and Greene [3] obtained almost twice as much total shoot elongation and a more luxuriant root system in plants supplied with vitamin B_1 than in similarly treated plants without vitamin B_1. According to Bonner,[4] vitamin B_1 is essential for root growth and in the presence of light it is normally formed in the leaves. The root of a normal seedling may receive its supply of this vitamin either from the green leaves or from the seed where it is normally stored in sufficient quantity for the needs of the young plant. The favorable behavior of the grafted mangosteen plants of Oliver might be attrib-

[1] Received for publication April 5, 1940.

[2] OLIVER, GEO. W. THE SEEDLING-IN IN MARCH AND NURSE-PLANT METHODS OF PROPAGATION. U. S. Bur. Plant Indus. Bul. 202, 43 pp., illus. 1911.

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[4] BONNER, JAMES. THE ROLE OF VITAMINS IN PLANT DEVELOPMENT. Bot. Rev. 3: 616–640. 1937. See pp. 628–630.

uted to a vitamin B_1 deficiency in the mangosteen seed being supplemented from a well-supplied root system or from the active leaves of the *Garcinia tinctoria* stock plant.

With knowledge of this and of the results of Bonner and Greene with *Aleurites fordii* as a background, the writer conducted an experiment at the Puerto Rico Experiment Station to determine whether supplementary growth substances may be obtained by mangosteen seedlings from a water extract of brewer's yeast to give the stimulation needed for satisfactory growth. Brewer's yeast is known to be a good source of vitamin B_1; however, this vitamin is not the only growth substance which roots can obtain from such yeast.

EXPERIMENTAL METHODS

Seeds were planted individually in cylinders 4 inches in diameter by 1 foot deep filled with dead sphagnum moss; the seeds were firmly embedded in the moss. Moisture was added only in nutrient solution. Fresh mangosteen seeds germinate slowly but without difficulty.

The experiment consisted of two treatments each containing 90 plants. All plants were irrigated once weekly with an excess of nutrient solution so that a flushing action was thereby accomplished. The nutrient solution used consisted of White's [5] solution with one-tenth of 1 percent of raw cane sugar to which were added the trace elements through a modification of Hoagland's mixture.[6] One of the two lots of plants received a water extract of brewer's yeast in addition to the combination of White's solution and Hoagland's A to Z mixture and raw cane sugar. The nutrient solutions were prepared in 18-liter quantities. Stock water extracts of yeast were prepared by heating for 96 hours, at a temperature of 100° to 103° C., crown-capped bottles each containing 7.2 gm. of Anheuser-Busch strain K yeast suspended in 200 ml. of distilled water. After standing for 3½ months, this preparation had a pH value of 5.17. Fifty milliliters of the cooled supernatant fluid thus obtained was used in the preparation of 18 liters of the nutrient solution for the yeast-fed plants.

The plants were grown for 7 months under uniform moistureproof protective sashes in the open nursery yard where uniform shade was provided by light cheesecloth. During the last 8 months of the experiment the plants were grown in a greenhouse where shade was provided by light cheesecloth. Temperatures were higher in the greenhouse than in the nursery yard.

EXPERIMENTAL RESULTS

The advance in development of the plants that received yeast extract, as illustrated by figure 1, *A*, over the plants that did not receive yeast, as illustrated by figure 1, *B*, distinctly shows the beneficial effect of yeast upon the development of the mangosteen plants during their first 10-month growth period. An increase in growth during this most critical period was obtained when the plants in

[5] WHITE, PHILIP P. POTENTIALLY UNLIMITED GROWTH OF EXCISED TOMATO ROOT-TIPS IN A LIQUID MEDIUM. Plant Physiol. 9: 585–600, illus. 1934.

[6] ROBBINS, WILLIAM J., and SCHMIDT, MARY BARTELY. GROWTH OF EXCISED ROOTS OF THE TOMATO. Bot. Gaz. 99: 671–728. 1938.

sphagnum moss irrigated with nutrient solution were given water extract of brewer's yeast. As leaf area increased, differences due to yeast-extract stimulation diminished. At the seedling age of 10 months the increase due to yeast extract was 75.8 percent, while at 15 months it was 46.8 percent. If the effective principle in the yeast extract was vitamin B_1, this decrease in growth rate was as should be

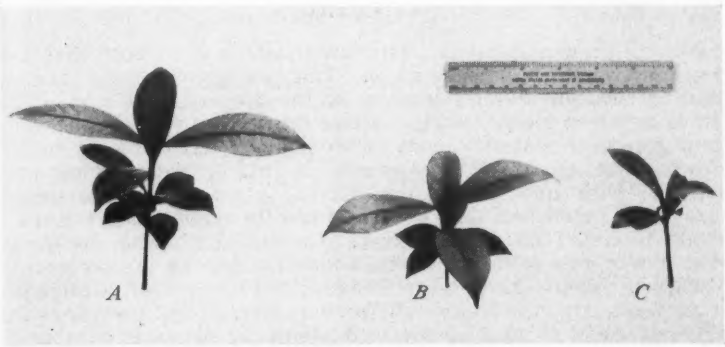

FIGURE 1.—Typical plants of *Garcinia mangostana* grown under different treatments. *A* and *B* were grown in dead sphagnum moss irrigated with nutrient solution. *A* received in addition a water extract of brewer's yeast. *C* is a plant of comparable age grown in garden soil only.

expected, for this vitamin is developed in the leaves and translocated to the roots. Table 1 presents the average leaf areas and the percentage increase for the two treatments together with the standard deviations and coefficients of variability for the measurements.

TABLE 1.—*Average leaf area and other data pertinent to the evaluation of differences between mangosteen plants grown with and without yeast extract*

Treatment	Age of plants	Average leaf area	Increase due to yeast	Standard deviation	Coefficient of variability
	Months	*Cm.²*	*Percent*		*Percent*
Nutrient solution	10	97.94		8.95	9.1
	15	252.18		109.34	43.4
Nutrient solution with yeast	10	172.20	75.8	12.23	7.1
	15	370.12	46.8	164.71	44.5

That the average leaf-area difference between the two treatments at both age levels was due directly to the effect of the addition of yeast is shown by the fact that this difference exceeded its standard error by an amount sufficient to assure an accuracy well beyond the 1-percent point where the odds are 99 to 1.

All the plants in this experiment, whether they received yeast extract or not, grew much more rapidly than those planted in good garden soil. The typically poor development of mangosteen plants grown in good garden soil is illustrated by figure 1, *C*, a plant from a

group not within this controlled experiment and a few weeks older than *A* and *B*. Whether the increase in growth of the plants grown in sphagnum moss and receiving only nutrient solution over those grown in soil was due to the nutrient solution or to the sphagnum moss or both was not brought out in this experiment. The profound increase in growth is, however, highly insignificant

SUMMARY

Normal growth in young mangosteen plants is so poor that few develop beyond the juvenile stage. This poor growth is the greatest limiting factor in the development of this tropical fruit crop. The few mangosteen plants that do survive the weak juvenile period continue growth to maturity much as do plants of any other vigorously growing tree species. Plants grown in dead sphagnum moss and irrigated with nutrient solution having yeast extract developed 75.8 percent more leaf area within 10 months than similarly treated plants receiving no yeast extract. Growth stimulation by yeast extract was greater in the first 10 months than in the succeeding 5 months. Such behavior should be expected if the effective principle in the yeast extract is vitamin B_1, for, normally, a plant provided with leaf area equal to that of these 10-month-old plants is capable of producing a sufficient quantity of vitamin B_1 for its current growth needs. Correlation is seen between this early stimulation by yeast extract and the growth-stimulating effect Oliver obtained when mangosteen was grafted on the more vigorous *Garcinia tinctoria*. Growth in all plants within this experiment whether or not they received yeast extract was profoundly better than that of plants grown in good garden soil only.

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